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SERUM LIPIDS AND FATTY ACID COMPOSITION OF SERUM LECITHIN IN THE NON PREGNANT STATE IN PATIENTS WITH PREVIOUS CHOLESTASIS OF PREGNANCY

Goran Samsioe

From the Department of Obstetrics and Gynecology (Head Prof S Brodus) Sahlgrenska Hospital University of Göteborg Göteborg Sweden

Abstract Serum lipids and fatty acid composition of serum lecithin were studied 8-21 months after delivery in 70 non pregnant women who during their latest pregnancy had presented signs of CP. These women were compared with normal non pregnant women ($n=18$) and with patients with cholestasis of pregnancy (CP) ($n=8$). In the non pregnant state women with previous cholestasis of pregnancy (CP) showed in serum lecithin a low relative content of palmitic (16:0) and linoleic (18:2) acids and a high portion of stearic acid (18:0). These findings indicate as a basic defect in CP a reduction in liver lecithin synthesis via pathway I (Kennedy's pathway) in favour of an increased pathway II (Greenberg's pathway). These data further support the hypothesis of increased estrogen activity as an etiological factor in CP. The basic influence by pregnancy on serum lipids and serum lecithin fatty acid composition was the same in women with CP as in women with a normal pregnancy.

Cholestasis of pregnancy (CP) occurring in certain disposed women is considered to be hormonally induced. Among the gestational hormones estrogen has been suggested to be responsible by provoking impaired hepatic excretory function (2). An exaggerated reaction to normal amounts rather than abnormally high serum levels of estrogen to be a likely mechanism (25).

Serum lecithin relative fatty acid composition in CP is characterized as compared with normal pregnancy by a low palmitic acid (16:0) and a high linoleic acid (18:2) content (11-13).

The influence of normal pregnancy on serum lecithin relative (and absolute) fatty acid composition are characteristic increases in palmitic (16:0) and oleic (18:1) acids and decreases in stearic (18:0), linoleic (18:2) and arachidonic (20:4) acids

The present paper deals with serum lipids and fatty acid composition of serum lecithin as determined by gas liquid chromatography (GLC) in non pregnant women with previous cholestasis of pregnancy studied 8-21 months after termination of their cholestatic pregnancy.

MATERIALS AND METHODS

Clinical series

Twenty eight women with cholestasis of pregnancy pregnant CP (mean gestational week 34.9 range 6-40 weeks) were studied. All patients presented a history typical for cholestasis of pregnancy and had immunologically detectable amounts of LPX in serum. Apart from the cholestatic syndrome all pregnancies were uncomplicated at the time of blood sampling.

According to an earlier definition (14-15) fourteen women were designated as pruritus gravidarum (PG), i.e. serum total bilirubin (1.7 mg/100 ml) and SGOT/SGPT (50 units/l) and fourteen as hepatitis of pregnancy (HP), i.e. total serum bilirubin above 1.7 mg/100 ml and/or SGOT and/or SGPT (50 units/l).

Twenty women with previously diagnosed cholestasis of pregnancy previous CP (mean age 7.0 years range 7-35 years) were re-examined 8-21 months after parturition. All patients were healthy and had regular menstruations and no women were nursing their babies at the time of the study. Pruritus had disappeared immediately after delivery and serum bilirubin and alkaline phosphatase values were normal. During their cholestatic pregnancy 9 women were designated as pruritus gravidarum (PG) and 11 as hepatitis of pregnancy (HP). No patients were using oral contraceptives.

Eighteen normal non pregnant healthy young women (mean age 6.2 range 19-34 years) with regular menstruation not using oral contraceptives served as a control series. Blood samples were drawn on the first or second day of the menstrual bleeding.

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Table III Mean differences (Δ) (mole per cent) in composition of major fatty acids of lecithin in women with previous pruritus gravidarum (PG) ($n=9$ mean age 24 range 20-30 years) previous hepatosis of pregnancy (HP) ($n=11$ mean age 28 range 20-34) versus normal non pregnant women ($n=18$ mean age 26.2 range 19-34)

=0.05 level			=0.01 level			=0.001 level		
			Non pregnant state Previous PG vs normal			Non pregnant state Previous HP vs normal		
PC	PC		PC	PC		PC	PC	
Δ		P	Δ		P	Δ		P
16:0	-1.5		-1.4					
16:1 (n 7)	+0.1	-	+0.1		-			
18:0	+0.3	-	+1.1		-			
18:1 (n 9)	0.0	-	+0.1		-			
18:2 (n 6)	-1.1	-	-2.9		-			
0:3 (n 6)	+0.6	-	+0.7		-			
20:4 (n 6)	+0.7	-	+0.6		-			
22:6 (n 3)	+0.1	-	+1.2		-			
18-22 (n 6)	+1.8	-	-0.4		-			
18:2/20:4	-0.6	-	-0.7		-			

14:0 15:0 18:3 (n 6) 18:3 (n 3) +2.0 1 (n 9) 2:4 (n 6) 22:5 (n 6) and 27:5 (n 3) have been identified but not tabulated as their concentrations generally were less than 1%. The fatty acid composition of phosphoglycerides was analysed but only the results obtained in lecithin (PC) are tabulated as the fatty acid composition of lecithin more readily explains the metabolic influences (77).

Table IV Composition of major fatty acids and mean differences (Δ) in women with cholestasis of pregnancy ($n=28$ week of gestation mean 34.9 range 26-40) and in non pregnant women with previous cholestasis of pregnancy ($n=70$)

Figures are given in mole per cent of methyl esters
=0.05 level =0.01 level =0.001 level

Cholestasis of pregnancy					Pregnant vs non pregnant	
Pregnant			Non pregnant		PC	PC
%	S.E.M.		X	S.E.M.	Δ	P
16:0	34.7	0.43	28.3	0.29	+6.4	
16:1 (n 7)	1.3	0.07	0.8	0.04	+0.5	
18:0	10.1	0.79	14.6	0.74	-4.5	
18:1 (n 9)	14.4	0.41	11.8	0.30	+2.6	
18:2 (n 6)	24.3	0.69	24.6	0.62	-2.1	
0:3 (n 6)	3.7	0.11	2.5	0.18	+0.7	
0:4 (n 6)	6.1	0.74	7.6	0.76	-1.5	
22:6 (n 3)	3.9	0.73	4.5	0.34	-0.6	
18-22 (n 6)	33.9	0.53	36.8	0.48	-2.9	
18:2/20:4	4.4	0.30	3.6	0.19	+0.8	
Fatty acid mg/100 ml	117	5.7	86	4.9	+31	

Statistical methods

Conventional methods were used for the calculation of means standard deviations and standard error of means Student's *t* test was used to evaluate differences between groups. Values of $p \leq 0.05$ were considered to be statistically significant.

RESULTS

A Non pregnant state Previous CP compared with controls

In serum lipids (Table I) no differences were found in women with previous CP on comparison with the control series of non pregnant women.

In the relative fatty acid composition of serum lecithin (PC) (Table II) in previous CP 16:0 (palmitic acid) ($p < 0.01$) and 18:2 (linoleic acid) ($p < 0.05$) were lower and 18:0 (stearic acid) higher ($p < 0.05$) than in the controls.

Yet in the subgroup of previous PG (Table III) 16:0 was lower ($p < 0.05$) than in the controls. In the subgroup of previous HP (Table III) all characteristic changes of previous CP were present i.e. 16:0 ($p < 0.01$) and 18:2 ($p < 0.05$) were lower and 18:0 higher ($p < 0.05$).

As the absolute amounts of lecithin (Table IV) were not different (191 and 198 mg/100 ml respectively) in the two groups these differences between previous CP and controls still persist when cal

Table V Composition of selected fatty acids and mean differences (Δ) in lecithin and calculated lecithin in women with cholestasis of pregnancy ($n=29$ week of gestation mean 34.9 range 26-40) and in non pregnant women with previous cholestasis of pregnancy ($n=20$)

Fatty acid in lecithin	Pregnancy	Non pregnant	Δ	Pregnancy vs non pregnant P
	Lecithin (mg/100 ml)	Lecithin (mg/100 ml)		
16:0	86.6	50.0	+36.6	0.001
18:0	27.8	28.5	-0.7	N.S.
18:1 (n 9)	39.2	27.7	+16.5	0.001
18:2 (n 6)	66.0	50.9	+15.1	0.001
20:4 (n 6)	17.8	13.8	+2.0	0.001
18-22 (n 6)	95.2	73.2	+22.0	0.001
Lecithin mg/100 ml	769	191	78	0.001

culated on absolute amounts of lecithin i.e. lecithin mg/100 ml with one particular fatty acid

B Previous CP compared with CP

All serum lipids (Table I) were lower in the non pregnant state (in women with CP) than during pregnancy (CP). Serum cholesterol was reduced after pregnancy by 77 mg/100 ml ($p<0.001$), serum triglycerides by 162 mg/100 ml ($p<0.001$) and calculated lecithin by 78 mg/100 ml ($p<0.001$).

In the relative fatty acid composition of serum lecithin (PC) (Table IV) in the non pregnant state (previous CP) 16:0 (palmitic acid) ($p<0.001$) and 18:1 (oleic acid) ($p<0.001$) were lower and 18:0 (stearic acid) ($p<0.001$), 18:2 (linoleic acid) ($p<0.001$), 20:4 (arachidonic acid) ($p<0.001$) and sum (n 6) ($p<0.001$) higher than in the pregnant state (CP).

Calculated as absolute amounts (Table V) i.e. lecithin in mg/100 ml with each particular fatty acid in previous CP 16:0, 18:1, 18:2, 20:4 and sum of (n 6) all were lower ($p<0.001$) than in CP.

DISCUSSION

Women with previous CP (studied 8-21 months after delivery) showed serum lipid values in agreement with those of healthy non pregnant women of the same age. The marked increase in serum lipids characteristically seen in CP was not found after delivery in the previous CP group.

Women with previous CP showed a low relative content in serum lecithin of palmitic (16:0) and linoleic (18:2) acid and a high proportion of stearic acid (18:0) as compared with the non pregnant

controls. The characteristic reduction in palmitic acid was also present in the subgroups designated as previous PG and previous HP.

The same influences by pregnancy as seen in normal women (22) on serum lecithin relative fatty acid composition were experienced when patients with CP were compared with women with a previous CP i.e. high content of palmitic (16:0) and oleic acids (18:1) and low portion stearic (18:0), linoleic (18:2) and arachidonic (20:4) acids.

In this comparison it should be pointed out that pregnancy in CP prone women causes a less marked increase in palmitic acid and a more pronounced increase in oleic acid concomitant with a lesser decrease in stearic and linoleic acids.

An increase in serum lecithin during pregnancy is most likely due to increased synthesis in the liver (1). In the liver lecithin is synthesised along different intracellular pathways. The major pathways are pathway I: cytidine diphosphate (CDP)-choline diglyceride pathway (Kennedy pathway) and pathway II: cytidine diphosphate (CDP)-ethanolamine methylation pathway (Greer's pathway). Pathway I is the faster and quantitatively most important (16, 20). This pathway preferentially gives in 1 position palmitic acid (16:0) and in 2 position linoleic (18:2) or oleic acid (18:1) (8). Pathway I in the liver is stimulated *in vivo* (in the rat) (3) and *in vitro* (human liver slices) (6) by the presence of bile acids.

Pathway II is the important source of lecithin containing in 1 position stearic acid (18:0) and in 2 position arachidonic acid (20:4) as well as other polyunsaturated fatty acids of longer chain length and greater unsaturation (e.g. 22:6) (7). In the

animal *in vivo* and *in vitro* studies indicate that pathway II is more active in the female (17-26) as well as during estrogen administration (18). Studies during the menstrual cycle in young women suggest that this influence by estrogens on pathway II is applicable also in man (10).

In a previous study (12) it has been shown that pregnant women in the early stage of CP hepatitis of pregnancy (HP) of short duration (before a major influence of raised bile acids on liver lecithin synthesis) have a characteristically high relative content of stearic acid (18.0) and arachidonic acid (20.4) in serum lecithin. These findings were interpreted as an indicator of an enhancement of liver lecithin synthesis through pathway II due to an increased influence of estrogens.

In the present study in non pregnant women with previous CP a high relative content of stearic (18.0) and arachidonic (20.4) acids in serum lecithin suggests that already in the non pregnant state of patients with previous CP (without pruritus and with normal liver function tests) an enhanced lecithin synthesis by pathway II is at hand. Simultaneously occurring low palmitic (16.0) and linoleic (18.2) acids suggest in addition a decreased activity of the liver lecithin synthesis through pathway I.

These differences in relative fatty acid composition were still present when calculated on an absolute basis, i.e. on lecithin (mg/100 ml) with each particularly fatty acid.

A high frequency of gallbladder disease (as verified by X-ray after pregnancy) in women with CP has been reported earlier (23). Gallstone formation is dependent on bile composition and lecithins have quantitatively and qualitatively been shown to be of importance in the solubility of cholesterol in bile. In women with a history of CP an altered liver lecithin metabolism might therefore be responsible for the formation of lithogenic bile.

The etiology of CP is unknown. An increased liver sensitivity to estrogen has been suggested to be an important contributory factor. The data presented suggest a basic defect present already in the non pregnant state of CP prone women reflected in liver lecithin metabolism, i.e. an increased pathway II and a decreased pathway I activity. *In vitro* and *in vivo* studies in the animal as well as preliminary data in man suggest that estrogen is one factor that enhances liver lecithin

synthesis through pathway II concomitant with a reduction of pathway I.

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UROGRAPHY AND RENOGRAPHY IN THE FOLLOW UP OF PATIENTS WITH TOXEMIA OF LATE PREGNANCY

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Abstract We have studied 129 patients with toxemia of pregnancy two months post partum by urography and renography. The same investigations were performed on 17 healthy parturients post partum. 49 patients (38.8%) had an abnormality either in urography or renography. 20 patients (15.5%) had an abnormal urogram and 42 (32.6%) an abnormal renogram. The findings correlated so that 7 patients had an abnormal urogram with a normal renogram and 13 patients had an abnormality in both investigations. 79 patients had only an abnormal renogram. In the control series 3 patients had an abnormal urogram and the same patients also had an abnormal renogram. We divided the studied patients into groups according to the clinical severity of toxemia. Urographic and renographic findings were divided evenly in the series so that abnormalities were no more common in the severe cases. Renography cannot be considered merely as a screening method when compared with urography in the follow up of toxemic patients, but these investigations are complementary.

If a patient with toxemia of pregnancy gives no history of previous hypertension or renal disease or if these conditions have not been observed during the first months of pregnancy, it is difficult to decide what sort of toxemia is present. The signs of toxemia during the first 24 weeks of pregnancy indicated a so-called superimposed toxemia. Superimposed toxemia can also be suspected in cases when the secretion of urine and electrolytes is increased during the night compared with daytime (10). Only a careful re-examination a few months post partum can ascertain the diagnosis. In addition to the routine investigations, such as recording the blood pressure and performing urine and blood tests, some special investigations, such as renal clearance tests, isotope renography and intravenous urography are of value (3, 5, 7). Renal biopsy and renal angiography are available but are somewhat more complicated. Using these investigations

it has been shown that severe toxemia may be associated with pre-existing renal or cardiovascular disorders in more than half of the cases (6, 7). These investigations suggest that the more carefully patients are examined, the less so-called pure or classic toxemia can be observed. Besides its theoretical significance, this matter is also of great practical importance because classic toxemia usually will be cured without leaving any signs (4), while hypertension and renal disease may become aggravated by toxemia of late pregnancy and perhaps finally lead to uremia and untimely death.

The purpose of this study is to investigate the occurrence of a possible renal abnormality in patients with toxemia by urography and renography. As well known, renography is a relatively easily performed investigation which upsets the patient only slightly. Both renography and urography can be done on outpatients. Further, the purpose of this study is to illustrate the correlation between these investigations and especially the use of renography as a possible screening method in the follow up of toxemia patients.

MATERIAL AND METHODS

The survey was carried out at Kuopio Central Hospital on patients who delivered between 1.11.1968-31.12.1971. The total number of deliveries during this period was 576. Toxemia (according to the American Committee on Maternal Welfare) occurred in 576 cases (10%) and the number of patients examined was 129 or 26% of the toxemia patients. These patients were divided before renography and urography into groups laid down by the American Committee on Maternal Welfare as modified by Werkö in 1948 (11) as presented in Table I.

To be able to compare the occurrence of a possible renographic or urographic abnormality with the severity of the toxemia, we placed the patients in each group

Table I Division of the patients into groups laid down by the American Committee on Maternal Welfare as modified by Werko in 1948

Clinical diagnosis	No of patients
I Mild pre-eclampsia	89
II Severe pre-eclampsia	29
III Eclampsia	1
IV Essential hypertension complicated by toxæmia of late pregnancy	10
V Renal disease complicated by toxæmia of late pregnancy	3
Total	129

order according to the clinical severity of toxemia. Our scoring system is designed only for putting these patients in such an order.

The criteria for severity were: onset of toxemia signs, duration of gestation at admission to hospital, duration of stay in hospital, duration of proteinuria, maximum protein excretion, average protein excretion, systolic blood pressure when discharged, average systolic blood pressure, average diastolic blood pressure, fetal weight, age of fetus and Apgar score. These criteria used for defining the severity of toxemia are presented in Table II.

Average value, maximum, minimum and standard deviation of those criteria are also shown in Table II.

For each criterion the patients got points: minimum 1 point and maximum 129 points (179 patients). The most severe case in each category got 1 point and the mildest case got 129 points. Thus the added points from all the 13 criteria determined the final order of the patients. Many of the patients got same total of points, so there were 96 different scores and the mildest case(s) was (were) there with the score of 96 in Fig. 1.

The patients were studied two months post partum by renography and urography.

The investigation methods for renography were as follows: (a) The labelled compound used was $^{99}\text{Tc}^{\text{m}}$ hippuran diluted with sterile isotonic saline solution to 0.5–1 ml. (b) The radiation detectors 1.75 x 2.54 in. (44 mm) were collimated so that the diameter of measuring area on skin was 10 cm and the distance of detectors from skin 19 cm. (c) Measuring technique: The measuring range 0–50 imp/sec, time standard 3 sec and the curve was recorded at a speed of 4 mm.

The investigation method in urography: As a control medium 20 ml Urografin[®] 60% (Schenck) i.v. A control radiograph from abdomen was taken before administration of contrast medium. The first exposure was made 1 min after injection and after this a compress was placed on lower abdomen. The following radiographs 10 and 15 minutes after the injection and after removing the compress a final radiograph from the abdomen.

The control series consisted of 17 patients who all had delivered in 1972. No signs of toxemia were observed in these patients and the deliveries were normal. They were also studied at their postnatal examination and both renography and urography were performed two months post partum.

RESULTS

Renographic findings are represented in Table III. In this study a total of 42 cases (32.6%) had obvious evidence of abnormal tubular function. The findings were either one-sided or bilateral and appeared either as a retardation of the secretory phase or as a prolonged renal transit time. Related to these there was possibly a spastic excretory phase or the excretion was gradual. This sort of excretory phase was considered to give certain evidence of a functional condition and not of a lesion of tubular function.

Table II Criteria used for defining the severity of toxemia and average, maximum, minimum and standard deviation of the criteria in the patients studied

No	Criteria	No of patients	Average value	Maximum	Minimum	SD
1	Onset of toxemic signs (weeks)	129	29.6	47.0	13.0	7.3
2	Duration of gestation at admission (weeks)	179	35.3	43.0	16.0	5.1
3	Stay in hospital (days)	129	26.5	104.0	9.0	15.7
4	Proteinuria (days)	129	20.5	58.0	3.0	11.8
5	Maximum protein excretion (g/24 h)	129	7.4	15.4	0.0	7.6
6	Average protein excretion (g/24 h)	179	1.2	43.0	0.0	3.8
7	Systolic blood pressure at admission (mmHg)	129	151.9	270.0	110.0	22.9
8	Systolic blood pressure when discharged (mmHg)	179	177.5	180.0	100.0	14.3
9	Average systolic blood pressure (mmHg)	129	136.7	174.0	101.0	12.7
10	Average diastolic blood pressure (mmHg)	179	89.4	108.0	57.0	9.4
11	Fetal weight (kg)	179	2.7	5.4	0.6	0.9
12	Age of fetus (weeks)	129	38.0	43.0	29.0	3.0
13	Apgar points	179	7.0	10.0	0.0	3.1

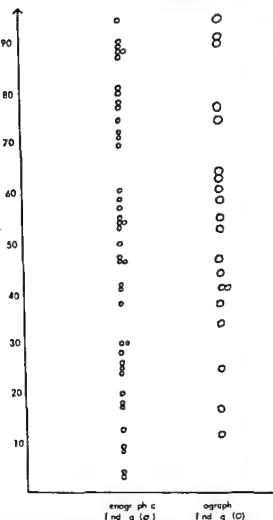


Fig. 1. Abnormal renographic and urographic findings in patients divided into groups according to the clinical severity of toxemia (most serious case = 1, mildest case = 96).

ion. There was one case with no secretion on one side and one case of hydronephrosis.

Urographic findings are presented in Table IV. 20 patients had abnormal urographic findings.

Table III. Abnormal renographic findings 2 months post partum in the toxemia patients studied.

Abnormal renographic finding	Mild pre-eclampsia	Severe pre-eclampsia	Eclampsia	Essential hypertension complicated by toxemia of late pregnancy	Renal disease complicated by toxemia of late pregnancy
pathological secretory phase (40)	7	9	1	1	1
silent kidney (1)					
hydronephrosis (1)		1			
total renographic changes (47)	27/86	10/79	1/1	1/3	3/10

(15.5%). There were 7 patients with movable kidney, 7 patients with morphological anomaly, 1 patient with evidence of early hydronephrosis, 1 patient with evidence of hydronephrosis and 2 of chronic pyelonephritis. One patient with silent nephrosclerotic kidney. One patient had a kidney with poor secretion which was evidence of nephropathy caused by diabetes, the primary disease in this patient.

Correlation between renography and urography. In the series of 129 patients, 47 patients had abnormal renographic findings and 20 patients abnormal findings on urography. Of these patients, 7 had an abnormal urogram with a normal renogram and 13 patients had abnormalities in both investigations. 29 patients had only an abnormal renogram. The relation between these findings is presented graphically in Fig. 1.

Fig. 2 shows the division of the findings in regard to the previously defined severity of toxemia. It can be observed that both abnormal urographic and abnormal renographic findings were divided quite evenly throughout the series.

In the control series of 17 patients, 3 patients had an abnormal renogram, which in all of them appeared as a pathological secretory phase indicating an obvious lesion of tubular function. All these patients had also an abnormal urogram. 2 had a movable kidney and the third a moderate morphological anomaly.

DISCUSSION

In the series of 129 patients, 42 patients had an abnormal renogram (32.6%) and 20 patients (15.5%) had an abnormal in urogram. 49 patients had abnormalities in either urography or renography (or in both). It was earlier known that 3 patients had renal primary disease and in addition 10 patients had essential hypertension. 116 patients were considered to have a pure toxemia, either a

Table IV Abnormal urographic findings 2 months post partum in the toxemia patients studied

Abnormal urographic finding	Mild pre eclampsia	Severe pre eclampsia	Eclampsia	Essential hypertension complicated by toxemia of late pregnancy	Renal disease complicated by toxemia of late pregnancy
Movable kidney (7)	3	3			1
Morphological anomaly (7)	5	1			1
Chronic pyelonephritis (2)	2				
Pre hydronephrosis (1)	1				
Hydronephrosis (1)					1
Poorly functioning kidney (1)				1	
Silent nephrosclerotic kidney (1)	1				
Total urographic changes (20)	17/86	4/29	0/1	1/3	3/10

mild pre-eclampsia severe pre eclampsia or eclampsia

In a previous study of 44 patients with toxemia Koskela and Pystynen (7) found a renal abnormality on urography in 14 patients (32%). Bedford et al (2) studied a series of 100 patients with toxemia by post partum urography and they observed an abnormality in 38 patients (38%). Compared with our study they had significantly more abnormal findings as result of urographic investigations. In our series an abnormal urogram was observed in 20 patients

(15.5%). 14 of these cases did not require repeated examinations (movable kidney morphological anomaly) by 6 patients repeated examinations were necessary.

Laakso has studied patients with toxemia by urography (8). The most important object of his study was the observation and follow up of tubular lesions in toxemia patients. Before delivery the secretion is retarded and after delivery when the condition of the patient improves the secretion usually also improves. In normal parturition secretion does not change during the pregnancy. Nieminen et al (9) observed that also in the second and third trimester of normal pregnancy both excretion and secretion can be retarded.

Bockler et al (3) found that 28 out of 77 patients with toxemia examined a few days after delivery had an abnormal renogram. In a re-examination 7-14 months post partum only 6 patients had a pathological renogram. In our study there was a significant number 12.6% of pathological urographic findings when examined 2 months post partum. Obviously findings of this sort are to a great extent reversible as Bockler et al has observed (3) and therefore their actual significance is difficult to evaluate.

In our study both the abnormal renographic and urographic findings are divided evenly in the whole series and e.g. no accumulation of pathological findings in patients with severe toxemia was observed as shown in Fig. 2.

Earlier Aantaa et al (1) have compared the relation between abnormal renographic and urographic findings in gynecological patients. Our study of these patients with toxemia lead to similar conclusions. The correlation between renographic and

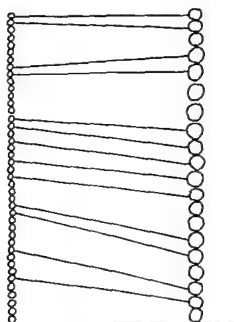


Fig. 2 Correlation of abnormal urographic and renographic findings. Abnormalities in the same patient are connected with a line.

urographic findings which has been earlier discussed shows that renography cannot be considered merely as a screening method when compared with urography but these investigations are complementary renography reveals disturbances in renal (tubular) function and urography shows the more severe morphological abnormalities and changes

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DECREASE IN THE MORTALITY RATES FOR LOW BIRTH WEIGHT INFANTS AFTER PHENOBARBITONE TREATMENT

Dyre Trolle

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Abstract A daily dosage of 100 mg phenobarbitone administered orally to pregnant women for three days or more immediately before delivery has reduced the various mortality rates for low birth-weight infants by 50% or more. Since it is hardly possible to treat all pregnant women in this way it is recommended to administer phenobarbitone to women with high risk pregnancies such as former stillborn infant, former premature delivery, pregnancy complications and planned induction of premature labour before the 37th week and until delivery takes place. If the mothers of low birth weight infants have not been adequately treated it is recommended that the newborn infant be given phenobarbitone injections immediately after birth and at 8-hour intervals. 10 injections in all.

In 1968 I published a preliminary communication (1) about a possible drop in the first week mortality rate for low birth weight infants after phenobarbitone treatment. The series was small and the difference was not statistically significant. In the meantime the series has been increased and now comprises 805 single born infants. The results are encouraging.

MATERIAL AND TREATMENT

We excluded all infants with lethal malformations and rhesus affected infants. 911 single-born infants weighing 500-3000 g were delivered in the obstetrical department during the period January 1 1968 to December 31 1971. All the mothers had been booked and controlled in ante natal clinic. More than one third (i.e. 30%) of the pregnant women received phenobarbitone viz 51 with pre-eclamptic toxæmia (according to our usual criteria) and 49 chosen at random. The drug was given orally the dosage was 50-200 mg per day and was continued until delivery took place. In addition 10 epileptic women received phenobarbitone all through the preg-

nancy. All the live-born infants except some in good health from the highest weight-class were transferred to the Department of Neonatology where every second infant was treated with phenobarbitone intramuscularly in the following way. The initial injection after birth and the following nine injections at 8 hour intervals. Each dose was 1 mg to infants of 1000 g or less, 2 mg to infants of >1000 to <1500 g, 3 mg to infants of >1500 to <2000 g, and 4 mg to infants of >2000 to <2500 g. It had been planned in advance that the initial injection should be given immediately after birth but in practice it was unfortunately not given until 5-15 minutes after and sometimes even 2 hours later. In some cases the midwives forgot to give the infants the initial injection and in consequence they were not treated at all.

106 infants (4 stillborn and 102 live born) born to mothers treated for less than 3 days (most often less than 4 hours) were excluded because we have found that it takes a couple of days before the phenobarbitone concentration in serum from a pregnant woman reaches 1 µg per ml and about a week before a fixed daily dose of phenobarbitone reaches a stable serum-phenobarbitone level. A daily dose of 50 mg or 100 mg of phenobarbitone results in a stable concentration of 3-6 µg per ml and 6-11 µg per ml respectively (investigations together with John Christiansen unpublished). In an earlier publication (24) it was shown that the phenobarbitone concentration in umbilical cord serum is 95% of the mother's serum value.

The reduced series comprises 805 infants i.e. 6% still born and 743 live born.

Autopsy on all the stillborn and neonatally dead infants was performed by the Laboratory of Paediatric Pathology. The reduced series was divided into two groups (Table I): (A) No administration of phenobarbitone to the pregnant woman and (B) Administration of phenobarbitone to the pregnant woman for three days or more immediately before delivery. In both groups it has been disregarded whether or not the infant had been given phenobarbitone.

The two groups differ only with regard to the number of women with pre-eclamptic toxæmia. In group A there were only 2% and most often mild cases at that. In group B there were 26% with many severe cases. Being aware that the two groups are not comparable I examined the outcome when all infants of women with pre-eclamptic

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24 hours in 23 of 40 i.e. 58% In group B none of the three dead infants died during the first 24 hours. The striking difference may be accidental and due to the small numbers.

All the dead infants had more or less pronounced atelectasis of the lungs. The causes of death are discussed later.

Respiratory distress syndrome (RDS) has been found in 70% of the first week deaths and in 14% of the live infants in group A. In group B the figures are 33% and 14% respectively. The incidence of RDS among all live born infants in group A is 17% and in group B 14%. These figures show that phenobarbitone treatment of the woman does not have any influence on RDS.

I have also examined the *first 4 weeks neonatal mortality rate* (first 28 days' deaths per 1000 live births) in order to see if the good results for the early neonatal mortality rate was due to a postponement of the time of death (Table V). Statistically the good results were less pronounced but the trend was still the same. The causes of death are discussed later.

Finally the *late perinatal mortality rate* (stillbirths + first 28 days' deaths per 1000 stillbirths + live births) is shown in Table VI. The results are encouraging as it appears from the statistical significance.

The influence of phenobarbitone treatment on the infant. In the above calculations it has been disregarded that alternate live born infants were treated with phenobarbitone. If group A is subdivided into subgroups (I) nontreated infants and (II) infants treated with phenobarbitone a comparison of the various mortality rates (early neonatal, first 4 weeks neonatal and late perinatal) between subgroups I and II shows that for all three rates sub-

Table IV *Early neonatal mortality rate per 1000 live births*

Weight-classes (g)	Group A	Group B	Value of $P=2\alpha$	Reduction (%)
$\geq 750 < 1000$	818	—	NS	
$\geq 750 < 1500$	386	59	< 0.01	
$\geq 750 < 2000$	206	29	$= 0.004$	
$\geq 750 < 2500$	73	15	$= 0.002$	80
$\geq 1000 < 1500$	305	67	NS	
$\geq 1000 < 2000$	164	29	< 0.005	
$\geq 1000 < 2500$	58	16	< 0.01	72
$\geq 1500 < 2000$	80	19	NS	
$\geq 1500 < 2500$	27	11	NS	59
$\geq 2000 < 2500$	13	8	NS	39

group II is lower (as shown in Fig. 1 for the weight class $\geq 750 < 2500$ g) but the differences are not statistically significant. The discrepancy between the numbers in the two subgroups i.e. 298 in subgroup I compared with 249 in subgroup II is due to the fact that in 24 cases the midwives forgot to give the initial injection of phenobarbitone and in consequence these infants were not treated at all. They survived the first 4 weeks of life and were included in subgroup I.

In group B the problem is more complicated. First it must be examined how the variation in dosage and duration of phenobarbitone treatment to the mother influenced the number of the first 4 weeks neonatal deaths. Table VII shows an even distribution of the neonatal deaths among the mothers who received 50 mg of phenobarbitone per day but not among the mothers who received 100 mg or more. The mortality for infants of the former was 6 out of

Table V *First 4 weeks neonatal mortality rate per 1000 live births*

Weight-classes (g)	Group A	Group B	Values of $P=2\alpha$	Reduction (%)
$\geq 750 < 1000$	818	—	NS	
$\geq 750 < 1500$	414	94	NS	
$\geq 750 < 2000$	279	100	< 0.03	
$\geq 750 < 2500$	80	41	< 0.08	49
$\geq 1000 < 1500$	339	333	NS	
$\geq 1000 < 2000$	189	103	NS	
$\geq 1000 < 2500$	65	41	NS	37
$\geq 1500 < 2000$	100	38	NS	
$\geq 1500 < 2500$	31	17	NS	45
$\geq 2000 < 2500$	13	8	NS	39

Table III *Number of pregnant women dosage and duration of treatment*

stillbirths in brackets

	Daily dose of phenobarbitone in mg			
	50	100	≥ 150	Total
1st				
< 7	16 (1)	11	8 (1)	35 (2)
< 14	18	28 (2)	7 (1)	53 (3)
2nd	26 (1)	62 (2)	28	116 (3)
3rd	60 (2)	101 (4)	43 (2)	204 (8)

no demonstrable cause of stillbirth

Table VI Late perinatal mortality rate per 1000 stillbirths+live births

Weight-classes (g)	Group A	Group B	Value of $P=2\alpha$	Reduction (%)
$\geq 750 < 1\ 000$	905	333	NS	
$\geq 750 < 1\ 500$	564	455	NS	
$\geq 750 < 2\ 000$	376	171	< 0.002	
$\geq 750 < 2\ 500$	163	78	< 0.003	52
$\geq 1\ 000 < 1\ 500$	466	473	NS	
$\geq 1\ 000 < 2\ 000$	318	164	< 0.02	
$\geq 1\ 000 < 2\ 500$	136	75	< 0.03	45
$\geq 1\ 500 < 2\ 000$	224	56	$= 0.008$	
$\geq 1\ 500 < 2\ 500$	89	33	< 0.02	63
$\geq 2\ 000 < 2\ 500$	49	23	NS	53

58 (average weight 1971 g) and for infants of the latter 2 out of 138 (average weight 2025 g). The causes of death were as follows. Col 1A 2 infants weight 1180 g and 1800 g both mothers had a severe pre-eclamptic toxæmia. Col 1B 2 infants weight 1300 g and 2170 g the former died from a sepsis which developed neonatally the latter had a subtotal atelectasis of the lungs but no RDS otherwise there was not any demonstrable cause. Col 1C 2 infants weight 1350 g and 1490 g both died because of a neonatally developed infection sepsis

and pneumonia Col 2B The infant weighed 1300 g the mother had a chronic hypertension and a severe abruptio placentæ Col 3C The infant weighed 1980 g died because of a neonatally acquired pneumonia. The higher mortality among infants born to mothers who received only 50 mg daily is statistically significant ($P=2\alpha < 0.02$).

Six out of 8 dead infants in Table VII were treated with phenobarbitone. The remaining 2 not treated infants were born to mothers who had received 50 mg daily.

The table seems to imply two important facts. Firstly that it is of no value to administer phenobarbitone injections to the infant if the mother has been given 100 mg of phenobarbitone or more daily. Secondly that the same good results can be reached by giving the mother 50 mg of phenobarbitone daily even if the infant is given phenobarbitone injections. In other words it is the infant's serum phenobarbitone concentration at birth which plays a decisive part in obtaining the good results.

A comparison of the various mortality rates between subgroup I, subgroup II and group B undertaken in the same way as shown in Tables IV and V shows that for each factor group B has the lowest rates as demonstrated in Fig. 1 for the weight-class

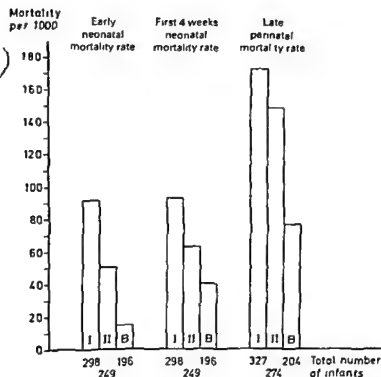


Fig. 1 The various mortality rates for weight class $\geq 750 < 2500$ g. I = phenobarbitone treated infants from group A II = phenobarbitone treated infants from group B group B. The difference between I and II is not statistically significant for any of the three mortality rates. The difference between II and B is statistically significant only for the late perinatal mortality rate ($P=2\alpha < 0.03$). The difference between I and B is statistically significant for all rates ($P=2\alpha < 0.001, 0.04$ and 0.001).

Table VII Number of pregnant women with live born infants Dosage and duration of treatment

Every second live-born infant was treated with phenobarbitone. The first 4 weeks' neonatal deaths are shown in brackets.

Days	Daily dose of phenobarbitone in mg			
	Col 1 50	Col 2 100	Col 3 ≥150	Total
A ≥3-<7	15 (2)	11	7	33 (1)
B ≥7-<14	18 (1)	26 (1)	6	50 (3)
C ≥14	25 (7)	60	78 (1)	113 (3)
Total	58 (6)	97 (1)	41 (1)	196 (8)

1. One of these did not receive phenobarbitone treatment.

≥750-<2500 g. Furthermore there is more often statistical significance between subgroup I and group B than was the case between group A and group B. On the other hand the statistical significance is less frequent between subgroup II and group B than was the case between group A and group B.

The causes of death for the infants who died during the first 4 weeks of life (Table VIII). In a number of cases there were pregnancy complications which contributed to the neonatal death. In subgroup I this was the case with 4 of 28 (i.e. 4.3%) viz. 1 with diabetes mellitus (White class C), 1 with placenta praevia and 2 with a mild abruptio placentae. The infants born to the three last mentioned mothers had RDS. In subgroup II there were 3 of 16 (i.e. 18.8%) viz. 1 with diabetes mellitus (White class D) and 2 with placenta praevia. These 3 infants had RDS. In group B there were 3 out of 8 (i.e. 37.5%) viz. 1 with chronic hypertension and a severe abruptio placentae and two with pre-eclamptic toxæmia, very low values of estriol in urine and more than half of the placenta was degenerated (white infarcts). None of these children had RDS.

In a number of other cases neonatal complications arose which in association with RDS caused the death. Thus in 5 infants in subgroup I pneumonia, pneumothorax and rupture of falx cerebri. The autopsy also showed that 2 of the infants had kernicterus. In subgroup II 5 infants had sepsis, purulent meningitis, pneumonia and rupture of the liver with haemoperitoneum. In group B 4 infants had sepsis and pneumonia, one of them had Down's syndrome as well.

The remaining 28 cases all died within the first three days except one who died 103 hours old. 18 of them had RDS. The post mortem examination showed various degrees of atelectasis of the lungs and in many hyaline membranes. The infants are distributed 19 in subgroup I, 8 in subgroup II and 1 in group B (whose mother had received 50 mg of phenobarbitone for 8 days but who had not received phenobarbitone itself).

Table VIII gives a summary of the causes of death. The big difference in the mortality between subgroup I, subgroup II and group B at no demonstrable complications etc. is immediately evident. There is statistical significance between I and B ($P=2\alpha=0.001$) between I and II+B ($P=2\alpha<0.005$) and between I+II and B ($P=2\alpha<0.004$).

The mortality rate in connection with pregnancy and neonatal complications in Table VIII is the same for the groups in question although it must be pointed out that in reality the result for group B is better because 76% of the mothers in this group—as mentioned above—had pre-eclamptic toxæmia against 2% in group A.

DISCUSSION

In the present series phenobarbitone administered orally to pregnant women for three days or more immediately before delivery reduced the various mortality rates by 50% or more. If the mothers have not been treated it seems that phenobarbitone given as injections to the infant is valuable. In this connection some questions concerning the drug will be discussed.

Table VIII Number of dead infants during the first 4 weeks

	Group A		
	Subgroup I (98 live born infants)	Subgroup II (49 live born infants)	Group B (19 live born infants)
Pregnancy complications	4	3	3
Neonatal complications	5	5	4
No demonstrable complications except low birth weight	19	8	1
Total	8	16	8

Risk of phenobarbitone treatment In a few cases it was necessary to stop the medication to the mothers because they developed a skin rash. Some mothers complained of being sleepy and lazy. Many times the latter disadvantage was caused by aversion to taking an old-fashioned sedative. If the tablets (100 mg daily) were called K 100 and the mothers were told that the drug was good for the child there were as a rule no complaints.

The average Apgar scores 1 minute after birth were 7.9 in Group A and 7.6 in group B. In group A 19% has Apgar scores 1 to 5, 25% 6 to 8 and 56% 9 to 10. In group B the percentages were 19%, 33% and 48% respectively. These findings are similar to those published by Donvig et al. in 1967 who compared the Apgar scores for two groups of infants delivered by Caesarean section. The same kind of anaesthesia was used but in one group the anaesthesia was started with a barbiturate enibomalum (250 mg or less intravenously). The enibomalum anaesthesia decreased the number of infants with the highest Apgar scores, increased the number of infants with the middle scores, while the number of infants with the lowest Apgar scores was unchanged. The authors concluded that the doped infants require careful attention after delivery, particularly in the form of artificial respiration. They also wrote that the Apgar score appears to be less useful as a prognostic indicator when enibomalum has been administered to the mother. In 1968 Secher & Wilhelm (29) were of the opinion that the use of barbiturates for induction in obstetric anaesthesia increases the survival rate for infants delivered by Caesarean section.

No side effects have been observed in the neonatal period. In particular there was no bleeding during the neonatal period as notified by Mountain et al. (25) and Evans et al. (12). Dortmann et al. (9) did not find any influence on the blood clotting factors. It is routine procedure in our department to give all infants 1 mg intramuscularly of phytomenadione immediately after birth.

In 1961 Brazelton wrote that in a group of children whose mothers received more than 150 mg of barbiturates as early as 1 hour and not more than 6 hours prior to delivery, there was a 5 days interval before 85% of their daily feedings were successful. This was in contrast to infants whose mothers received 60 mg of barbiturates or less or nothing at all where there was a 4 days interval. The usual period of disorganization (24 to 48 hours) seemed to be

prolonged for 1 to 2 days if the mothers had received 150 mg of barbiturates or more. Similar observations have not been made in our department but then the problem has not been systematically examined.

Desmond et al. wrote in 1972 that withdrawal symptoms starting from 30 minutes to 10 days postpartum and lasting for 2-6 weeks occurred in newborn infants whose mothers had been given daily dose of from 90 to 120 mg of phenobarbitone for at least 12 weeks prior to delivery. The infant had poor postnatal weight gain as well as hypercatability and restlessness. Although they appeared voraciously hungry, feeding was frequently followed by gagging and vomiting. Similar dramatic symptoms were never observed in our department.

The matter of congenital malformation as a consequence of phenobarbitone treatment to pregnant epileptics has been taken up during recent years (24, 23). The general opinion is that the probability of having a malformed child (most often cleft lip with or without palate) appears to be two to three times greater in epileptic women receiving anticonvulsant drugs. Untreated epileptic mothers have a malformation rate not significantly different from the control population (survey by Hill). Other authors find, however, that the matter has not been fully clarified as the articles published so far do not give sufficient information on the degree of epilepsy in treated and non-treated mothers (30).

In the present material administration of phenobarbitone was not started until the last 10 weeks of pregnancy, except to epileptic women.

Benefit of phenobarbitone treatment In addition to the favourable effect on the infant mortality rate the phenobarbitone treatment presents other advantages. In two papers published in 1968 (31, 35) I have shown that administration of phenobarbitone to the pregnant woman or to the newborn or to both reduces the incidence of infants with hyperbilirubinaemia (≥ 10 mg per 100 ml). The favourable result has been confirmed by seven authors (21, 33, 26, 40, 10, 32, 38, 11, 13, 41, 42, 9, 15).

Other authors (22, 6, 19, 31) have reported that they have been unable to find any such effect of phenobarbitone but here it should be taken into consideration that they did not begin treatment until the infants had become jaundiced and that the was administered orally.

Action of phenobarbitone Hepatic micros

enzymes situated in the smooth endoplasmic reticulum of the liver cells are responsible for the metabolism of endogenous and exogenous compounds. Administration of various drugs can stimulate the quantity and activity of these enzymes so-called enzyme induction. Remmer (27, 28) and Conney (4) have independently discovered that barbiturates are enzyme inducers (5). This induction increases the hepatic microsomal enzyme uridine diphosphoglucuronyl transferase responsible for the glucuronide conjugation of bilirubin (3). Recently it has been shown (16) that phenobarbitone given to the mother before delivery or to the infant from the time of birth enhances bilirubin clearance in the newborn infant.

Another favourable effect of phenobarbitone should be mentioned. Animal experiments have shown that the survival time of hypoxic animals previously anaesthetized with different barbiturates is longer than that of unanaesthetized animals exposed to the same low oxygen concentration. Barbiturates cause a reduction in the oxygen uptake by cerebral tissue. This depressant effect of barbiturates on cerebral oxygen consumption causes a greater resistance to hypoxia so that the tolerance of the brain is increased (39).

Whether other factors are involved in the reduction of the infant mortality rate is observed by us as hardly be decided at the moment.

Nor can it be decided whether phenobarbitone is the best possible barbiturate and perhaps quite different drugs may be a possibility. Thus Thompson et al. (34) showed that dicophane could reduce the plasma bilirubin of a 17 year old boy with familial unconjugated non haemolytic jaundice.

CONCLUSION

Since only 5 to 10% of pregnant women give birth to low birth weight infants it is an unrealistic thought that phenobarbitone should be given to all pregnant women during the last three months of pregnancy. But to my mind it would be a good idea to treat high risk pregnancies as for instance women with a history of a former stillborn infant, former premature delivery, pregnancy complicated especially pre-eclamptic toxæmia and hypertension and planned induction of labour before the 37th week. If treatment has been given during the last three days or more before delivery it would be unnecessary to treat the infant. If the

mother has not received phenobarbitone or received it for less than three days before delivery or administration was stopped prior to the last three days of pregnancy it is recommended to treat the infant immediately after birth and during the next three days of life.

The dosage to the mother should as a rule be 100 mg per 24 hours. For the infant the dosage recommended is mentioned above under Maternal and Treatment.

It is possible that the mortality rates could be still more improved by a supplementary use of betamethasone therapy to the pregnant woman as proposed by Liggins & Howie (20) who have found a favourable effect on RDS by using this therapy.

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BLOOD FLOW IN MYOMATOUS UTERI AS MEASURED BY INTRA ARTERIAL $^{133}\text{XENON}$

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Abstract Uterine blood flow has been studied using an arterial $^{133}\text{Xenon}$ Compartment analysis was used calculation of blood flow from the wash-out curves. In myomatous uteri the mean blood flow was 11.6 ± 1.70 ml min⁻¹ 100 g. In 8 control cases the mean blood flow was 20.1 ± 2.27 ml min⁻¹ 100 g. The difference is statistically significant on the 1% level. From the present study it cannot be decided how the uterine blood flow is distributed between myomata and surrounding myometrium.

The vascularization of uterine myomata has been the subject of several studies. The well known tendency in these tumors to undergo degenerative changes has been regarded the result of insufficient blood flow.

Myomata also have an influence on the vascularity of the surrounding myometrium probably thereby also affecting blood flow in other parts of the uterus. Abnormal uterine bleeding in connection with myomata has been ascribed to such vascular effects.

Injecting radio-opaque medium into surgically removed uteri Sampson (8) found varying vascularity in myomata depending on the size of the tumor. Small tumors were usually found less vascular than larger ones. Age was also found to influence the vascularity of uteri and myomata of older women showing lesser vascularity than those of younger women. In degenerating myomata a varying picture was found. Holmgren (6) using a similar technique described different types of degenerative changes. He stressed the difference between the hydropic degeneration rich in vessels and the dry necrosis poor in vessels. Faulkner (7) could not confirm this difference. He found as a rule a very rich arterial blood supply but very few veins in the myomata. The

venous network never reached the density of that of normal myometrium.

Fernstrom (3) used pelvic arteriography and in his radiographs he could not differentiate between arteries in the capsule and within the myoma naming them all tumor vessels. The density of tumor vessels was related to the size of the tumor. Smaller myomata had very few or no blood vessels and larger myomata were as a rule richer in tumor vessels.

Farrer Brown et al. (1) used a micro-radiographic and histological technique and found that the intrinsic blood supply of a myoma had no regular pattern. The arterial network was found to be less dense than in the surrounding myometrium. They did not mention myomata richer in arteries than the surrounding myometrium. The venous pattern was even more sparse than the arterial.

Studying the influence of myomata on the total uterine vasculature Sampson (9) found that some myomata were associated with a dilatation of uterine veins. He interpreted this to be the result of increased arterial inflow caused by the myomata. The association between excessive uterine bleeding and changes in the myometrial and endometrial venous plexuses is pointed out by Farrer Brown et al. (1). By compressing veins in their surrounding myomata cause congestion. When congestion occurs in veins draining the endometrium abnormal uterine bleeding easily results.

From morphological studies it is thus obvious that uterine myomata affect uterine blood flow by compressing surrounding vessels and by deriving their blood from branches of the uterine artery.

Information on intrinsic arteries seems contradictory. From available morphological data it is

difficult to draw any conclusions concerning the amount of blood flowing through myomatous and myomatous uteri. The only study giving quantitative values for blood flow in myomatous uteri known by the author is the work by Klingenberg (7).

Using an electromagnetic flowmeter placed around one of the uterine arteries he reports blood flow in 10 cases, 9 of which were myomatous. He found rather constant values irrespective of uterine weight, i.e. the heavier the uterus the lower the blood flow per unit weight. He suggests that this is the result of lower blood flow in myomatous than in myometrium.

Since very little quantitative data on blood flow in myomatous uteri exist it was considered of interest to apply the isotope clearance method to elucidate the conditions further.

METHODS AND MATERIAL

The theory of the isotope clearance method and the technical details in applying it to the study of uterine blood flow has been described in previous papers (4, 5). The intra-arterial injection technique described there was used. Conventional graphic separation of curve components was performed. These components were then used for the calculation of total blood flow within the isotope-labelled region. This value is in the following called uterine blood flow (UBF) and has been calculated according to eq. 1.

$$UBF = \frac{100}{\sum \frac{A_i}{F_i}} \quad (1)$$

A_i stands for percentage isotope in each compartment when elimination starts. F_i stands for blood flow in each compartment calculated from curve half times and expressed in $ml \cdot min^{-1} \cdot 100 g^{-1}$.

The percentage weights of compartments have been calculated according to eq. 2.

$$W_i = \frac{\frac{A_i}{F_i} \cdot 100}{\sum \frac{A_i}{F_i}}$$

where W_i stands for percentage tissue weight per unit blood flow F_i . When three curve components identified the percentage weight of the well perfused portion of tissue has been calculated according to the following modification of eq. 2.

$$\text{Percentage weight of well perfused tissue} = \frac{\left(\frac{A_1}{F_1} + \frac{A_2}{F_2}\right)}{\frac{A_1}{F_1} + \frac{A_2}{F_2} + \frac{A_3}{F_3}}$$

In all calculations of blood flows a partition coefficient of 0.7 was used.

Two groups totalling 15 patients were studied. In cases a total abdominal hysterectomy was performed. The first group included 7 patients with uteri containing multiple myomata. In all these cases the indication for surgery was uterine myomata causing abnormal uterine bleeding, symptoms of compression.

The other group of 8 patients was used as control group. This group was heterogeneous as to diagnosis and indication for surgery. Patients with one single myoma not exceeding 5 cm in diameter were accepted in this group. No case of cancer or precancer of the endometrium were included in either group.

The results are given in the text as mean \pm standard error of the mean. For statistical evaluation the Wilcoxon rank sum test was used.

Table 1 Myoma cases

Patient no	Age	Pregnancies	Phase of cycle	Blood flow in $ml \cdot min^{-1} \cdot 100 g^{-1}$			Well perf. tissue	Remarks
				UBF	Quick comp	Slow comp		
1	37	1-0-0	Prolif	10.5	3.7	6.5	15.8	
6	57	2-1-0	Prolif	7.1	13.9	6.1	13.3	
12	41	1-1-0	Prolif	14.6	34.1	10.1	13.2	
13	46	-1-0	Secr	15.6	30.3	4.7	43.3	
14	50	3-1-0	Secr	17.1	34.7	4	47.5	
19	43	2-0-0	Prolif / Secr	12.7	5.5	4.4	30.3	
24	39	1-2-0	Secr	1.5	16.2	2.1	9.9	
Mean	44.0			11.6	28.1	5.3	24.1	
S.E.M.	1.95			1.70	3.69	0.89	5.06	

Very quick comp.
16.7 $ml \cdot min^{-1} \cdot 100 g^{-1}$
* Kr instead of ^{51}Cr

First figure: number of deliveries; second figure: number of abortions; third figure: number of extrauterine pregnancies.

Table II Control cases

Patient no	Age	Diagnosis	Pregnancies ^a	Phase of cycle	Blood flow in ml min ⁻¹ 100 g			Well perf tissue	Remarks
					UBF	Quick comp	Slow comp		
5	35	Menorrhagia	4-0-0	Secr	19.7	4.5	10	30.4	
9	37	Menorrhagia	3-0-0	Oral contraceptives	14.9	3.4	4.1	38.3	
5	33	Endometriosis Dysmenorrhoea	2-0-0	Secr	2.1	40.4	7.8	27.9	Very quick comp 121 ml min ⁻¹ 100 g
6	44	Menorrhagia	3-1-0	Prolif	18.9	40.4	6.3	36.9	
7	38	Meno-metrorrhagia	2-0-0	Prolif	36.1	24.2	3.9	41.6	Very quick comp 3.5 ml min ⁻¹ 100 g
1	46	Meno-metrorrhagia	2-0-0	Prolif	17.2	30.3	9.3	49.3	
2	43	Vanocoele pelvis	3-0-0	Prolif	15.7	3.3	3.5	17.8	Very quick comp 179.7 ml min ⁻¹ 100 g ⁻¹
3	43	Ca cervix	2-0-0	Secr	16.1	32.3	6.2	37.8	
mean	39.9				20.1	34.3	6.4	32.5	
S.E.	1.46				2.27	2.02	0.82	3.36	

First figure number of deliveries second figure number of abortions third figure number of extrauterine pregnancies

RESULTS

The cases are presented together with results in tables I and II. In all cases multi-exponential wash-out curves were obtained. In three cases in the control group and one case in the myoma group an initial very quick elimination with a half time of 14-40 min was observed. The other two curve components showed small differences between the groups, the mean blood flow corresponding to the quick component being 28.1 ± 3.69 ml min⁻¹ 100 g⁻¹ in the myoma group and 34.3 ± 2.02 ml min⁻¹ 100 g⁻¹ in the controls and for the slow components 3 ± 0.89 and 6.4 ± 0.82 ml min⁻¹ 100 g⁻¹ respectively. The groups are not statistically separated on the 5% level.

When percentage tissue weights corresponding to the quick and very quick components are added the value in the myoma group is $24.1 \pm 5.06\%$ and of the controls $32.5 \pm 3.36\%$. The difference is not statistically significant. UBF calculated according to eq 1 was 11.6 ± 1.70 ml min⁻¹ in the myoma group and 20.1 ± 2.27 in the control group. This difference is statistically significant ($p < 0.01$).

^a If the groups are subdivided according to phase of

menstrual cycle no clear pattern is seen. There is a slightly higher mean age in the myoma group. This difference, however, is not statistically significant.

DISCUSSION

Uterine blood flow is probably affected by a multitude of factors. Examples of such factors are age, hormonal status, parity, uterine contraction, local infections and tumors, general and local hemodynamic factors such as arterial and venous blood pressure. This study concentrates on one of these factors—the effect of uterine myomata. However, the other factors are affecting the result of the study causing a considerable spread of values.

In the present method for study of uterine blood flow, the whole organ is probably not labelled with isotope. The isotope injection is made into the right uterine artery. There exist numerous anastomoses with the contralateral side but in all probability the right half of the uterus is preferentially labelled. It must thus be pointed out that the UBF is in fact blood flow in the region labelled with isotope. The conclusions concerning the flow differences between myoma and control cases are not affected by

A PROSPECTIVE STUDY OF DRUGS AND PREGNANCY

1 Psychopharmaca

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Abstract. A prospective study has been performed on the use of psychopharmaca during 6376 pregnancies and its relation to certain socio-medical variables and pregnancy outcome. An association between drug use and either miscarriage or legal abortion was demonstrated and conceivably due to an increased use of such drugs in wanted pregnancies. No indication of an association between drug use and birth of a dead or defective infant is found. Pregnancies where psychopharmaca were used during the first trimester had on average a longer duration than pregnancies where such drugs were not used but birth weight of full term infants was not affected.

Experimental studies on laboratory animals and extensive clinical experience, especially the thalidomide tragedy, have taught us that drugs and other chemicals when given to the maternal organism can damage the embryo. In well controlled animal studies such effects can be relatively easily demonstrated. They can be recorded by one or more of the following parameters: rate of major and minor structural malformations of the young, rate of stillbirth or neonatal death, miscarriage rate, weight of live born young. Similar effects should be looked for in the evaluation of possible embryopathic effects of drugs on the human embryo and fetus. Such analysis will, however, be complicated by many sources of variation. In a well planned animal experiment the distribution of individuals to groups receiving the drugs in various dosage or serving as controls is randomized. This is not the case in clinical studies. The presence of diseases or complaints affecting the administration of drugs distinguishes drug consuming and the control groups. Other

factors may also influence drug usage, e.g. social class, civil status, age, psychological factors.

The mere ascertainment of the fact that a certain woman has used a specific drug during a certain part of her pregnancy presents difficulties. Retrospective data on drug consumption, obtained after the end of the pregnancy, are liable to bias. A woman who has had a dead or malformed child or has experienced an unwelcome miscarriage has a stronger reason to search her memory for events which could explain the disaster, e.g. drug usage, than a woman who has had a normal living child. To a certain degree these fallacies can be balanced by close scrutiny of written records, including prescriptions. An elegant study by Nelson & Forfar (11) has used this technique. Another method is to only use information collected prospectively. False information may be included but data will be little biased by outcome of the pregnancy. Such prospective studies must necessarily result in much smaller series of malformed infants than a retrospective study can. This can be partly balanced by using other parameters like miscarriage rate and birth weight for the evaluation of drug effects. Recent publications on prospective studies on drug effect are those of Crombie *et al* (3) and especially Vilumsen (14). The latter study was performed on 9006 hospital births in Copenhagen and discusses—among many other features—drug use in relation to malformation of the infant.

Special interest has been paid to psychopharmaca in this connexion. Thalidomide, the best known and perhaps strongest teratogenic drug active on hu-

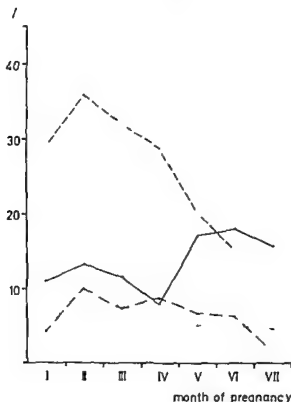


Fig. 1 Percentage women using psychopharmaca during different months of pregnancy according to pregnancy outcome — = miscarriage --- = induced abortion = live infant - - - = dead infant

mans belonged to this category psychopharmaca pass the blood-brain barrier and therefore probably also the placental barrier. These drugs are often used in early pregnancy and their use is not always justified by the signs or symptoms for which they are prescribed. New interest was stimulated in this group of drugs when McBride (9) claimed that an antidepressive drug imipramine was markedly teratogenic for humans. Many authors have contradicted that conclusion but some support was given by Idanpa-in-Heikkilä & Saxen (5) for at least a weak teratogenic action of tricyclic antidepressive drugs.

The present paper is the first in a series describing results from a prospective study on drugs and pregnancy. The data were collected during 1963-65. Later contributions will discuss anti-emetic drugs, hormones, and a number of miscellaneous drugs.

MATERIAL AND METHODS

Some information on the present study has been given earlier (4, 6, 7). The material is based on questionnaires

filled in by pregnant women in the city of Malmö during 1963-65. Every woman who was found to be pregnant was asked to complete a questionnaire which was returned within a few days. In this questionnaire information for the last menstrual period up to the first visit to the doctor was given. A new questionnaire was then handed over to cover the period up to her next visit to the doctor and so on—the entire pregnancy was usually covered by four such questionnaires. Part of the information is thus retrospective because it was given after the event but it is usually collected before the outcome of the pregnancy is known.

All gynecologists in Malmö inside and outside the hospital were engaged in this study. Information obtained from the questionnaires was supplemented with data from hospital records.

A total of 6 913 pregnancies were recorded but only 6 376 could be followed (92%). The outcome of the pregnancy was evaluated as miscarriage (up to the 6 gestational month), 448 induced abortion (legal or non-criminal abortions), 158 extra-uterine pregnancy, and birth of a child 5 753.

All living children were carefully investigated by a pediatrician after birth and were followed to about one year old at the child health centres. Autopsy was performed on infants and the age at death was recorded. The prevalence of major and minor malformations was recorded for all infants (4). Birth weight and placenta weight were also recorded.

Information on drug use was obtained mainly from the above mentioned questionnaires, sometimes supplemented from hospital records etc. The (approximate) time of intake of each drug used was recorded and referred to the gestational month of the pregnancy.

On the first questionnaire the woman was asked if the pregnancy was wanted or unwanted. Records of major and minor diseases were kept. The woman's age, parity, civil status and social class were recorded. Social class was evaluated by the health insurance class of the woman or her husband, whoever had the higher. As health insurance is compulsory in Sweden and the sum paid per day is proportional to the person's income, this gives an estimate of her socio-economic level.

RESULTS

Spontaneous and induced abortion

The frequency of women reporting to have used psychopharmaca during the various gestational months (up to and including the seventh) is shown in Fig. 1 in relation to pregnancy outcome. Women who will later miscarry report a higher rate of psychopharmaca use during the first months of pregnancy than do women whose pregnancies end at the birth of an infant, dead or alive. During the first month, i.e. before the woman knows she is pregnant, 42 of the 417 women who will later miscarry report that they used psychopharmaca. In

Table I Frequency of diagnosis of psychoneurosis

Outcome of pregnancy	No of diagnosis	Total no of women	% of women with diagnosis	χ^2
Spont abortion	10	448	2.2	8.7
Induced abort	78	158	17.7	35.4
Childbirth	48	5753	0.8	—

Comparison with women who gave birth to a child

control group of all women who bore a child 328 among 5753 used psychopharmaca. This difference is highly statistically significant ($\chi^2=13.1$ at 1 d.f. $P<0.001$). During the next few months there is still a statistically significant difference ($\chi^2=10.7$ and 6.2 $P<0.001$ and 0.02 $P<0.01$ resp.). After the fourth month there is a sudden increase in psychopharmaca consumption among women who will miscarry. There are only 81 women left in this group during the fifth month but 14 of these report use of psychopharmaca. This differs with high statistical significance from the rate among women who will bear a normal child—301 among 5753 such women used psychopharmaca during the fifth month of gestation ($\chi^2=22.7$ $P<0.001$).

Women who will later have an induced abortion show an increased rate of psychopharmaca consumption especially during early pregnancy. There is a marked decrease during the fifth and sixth months but relatively few women enter this group at that time. During the fifth month 98 women will still have an induced abortion and 20 report the use of psychopharmaca during that month. When compared with the figures for the third month (39/122) the difference appears to be marginal ($\chi^2=1.7$ at 1 d.f. P near 0.05).

Psychopharmaca use and medical and social variables

The most obvious disease resulting in a prescription of psychopharmaca is psychoneurosis. Table I gives the frequency of this diagnosis among women who will have a spontaneous abortion, an induced abortion or give birth to a child. As is seen from this Table there is a significant increase in the incidence of this diagnosis among women who will have a later abortion—whether spontaneous or induced. The latter is quite reasonable; the former may be due to the fact (7) that women who will have a spontaneous abortion to a higher degree

experience unwanted pregnancies than women who will have a child.

Psychopharmaca are prescribed and used also in the absence of a manifest psychoneurosis. One possibility is that a woman who chances to have an unwanted pregnancy would be more prone to use a sedative or an antidepressant drug. Table II shows in analysis of this phenomenon. A statistically significant overrepresentation of psychopharmaca usage during early pregnancy is obviously present among women with an unwanted pregnancy. Such a pregnancy is apt to end with an induced abortion—some of which may be taken as spontaneous—which will result in an apparent association between drug consumption and miscarriage. This phenomenon resembles that found earlier (7) on the relationship between smoking and abortion.

The desire for pregnancy is associated with a number of other characteristics; the most obvious one being civil status. A comparison was made of psychopharmaca usage during early pregnancy in women who ultimately bore a child. 4015 were married at LMP and 844 were unmarried. In the former group (92% of these women reported a wanted pregnancy) 14.9% used psychopharmaca during the first trimester but in the latter group only 12.7% did so. This difference is not statistically significant ($\chi^2=2.8$ at 1 d.f.) but at any rate the marked difference in percentage of unwanted pregnancies is apparently counterbalanced by other differences between the two groups of women which influence use of psychopharmaca.

Fig. 2 shows the use of psychopharmaca in women belonging to different social classes as judged by income. There is a slight trend to an increase in drug use with income class but this is of limited significance. A test of the trend in the frequency table according to Maxwell (8) gives a $\chi^2=3.9$ for first trimester psychopharmaca consumption ($P=0.05$) and $\chi^2=2.2$ for total psychopharmaca consumption (N.S.). This correlation is, however, also

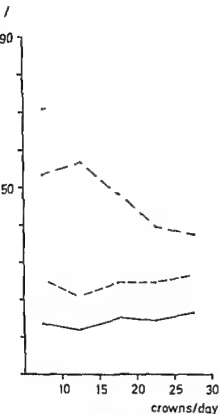


Fig. 2 The relation between some variables and social class estimated as crowns per day in health insurance for woman or her husband: whoever had the highest sum — = first trimester drug 777 = second third trimester drug — · — = wanted pregnancies — — = smokers

influenced by the desire for pregnancy. As is seen in this Figure, the percentage of wanted pregnancies is higher in the higher income groups than in the lower. Therefore a decrease in psychopharmaceutical use would be expected instead of the possible slight increase recorded. With a knowledge of the percentage of wanted pregnancies in each social class and the mean percentage of women using psychopharmaceuticals (Table II), the expected number of such women in each social class can be calculated, supposing no other factor influences drug use. The following result is obtained: class 0–10 113 expected 93 found; class 11–15 116 expected 85 found; class 16–20 226 expected 225 found; class 21–25 169 expected 170 found; and class 26–30 128 expected and 142 found. A χ^2 analysis gives $\chi^2=13.7$ at 4 d.f. $0.01 > P > 0.001$. Thus there is a relative increase in the use of psychopharmaceuticals when the changes in degree of desire for pregnancy are taken into consideration in the higher income groups. This phenomenon may be the same as that recorded for civil status. By contrast there

Table II Use of psychopharmaceuticals before the 4th gestational month and desire for the pregnancy

Desire for pregnancy	Total no of women	No of women using psychopharm.	Percentage women using psychopharm.
Wanted	5 136	651	12.7
Not wanted	1 087	253	23.3
Not stated	159	—	—

$$\chi^2=81.2 \text{ at } 1 \text{ d.f. } P<0.001$$

is a marked decrease in smoking frequency which reflects the changes in frequency of unwanted pregnancies.

The mean age of all women using psychopharmaceuticals during the first trimester is 28.04 ± 0.19 years, that of all women who do not report psychopharmaceutical consumption is 26.21 ± 0.08 . This difference is statistically highly significant ($t=8.9$, $P<0.001$). The mean age of women using barbiturates during the first trimester is 28.0 years, that of women using meprobamate is 27.9, and that of women using hydroxyzine is 29.6 years.

Social class and age are also associated. The mean ages in different social classes are: 0–10 24.26 years, 11–15 22.61 years, 16–20 26.78 years, 21–25 28.47 years, 26–30 30.76 years. With a knowledge of the number of women using psychopharmaceuticals and belonging to the different social classes, the expected mean age of all women using these drugs can be calculated and is found to be 26.9 years, which is still below the actual mean age of 28.04 ($t=3.9$, $P<0.001$). It thus appears that desire for pregnancy, age, social class and/or civil status all influence use of psychopharmaceuticals during early pregnancy.

Malformation rate

Fig. 3 presents the use of psychopharmaceuticals during different months of pregnancy, comparing women who gave birth to non-malformed infants, to infants with minor malformations only, and to infants with major malformations. Fig. 4 compares in a similar way two groups: normal infants and infants dead or malformed (=perinatal pathology). No obvious difference exists between the different groups—although the total perinatal pathology group appears to lie below the group of normal infants, though this is not statistically significant.

Table III compares the three different groups.

from another aspect incidence of psychopharmaca use at any time during the first trimester. The incidence of gross major malformations is slightly higher than in the control group but this difference can well be random ($\chi^2=1.0$ at 1 d.f. N.S.). The two other groups of malformations (minor and insignificant major) are even slightly decreased. The Table also summarizes the distribution of pregnancy outcome after the use of some specific psychopharmaca—alone or together with other drugs. The number of infants born with defects lies well within expected limits. The actual numbers are small however. Even for meprobamate which was used during the first trimester by 263 women who later had a child the recorded number of infants with major malformations could be a random minus variation at a major malformation rate of 1.72%—that is double the control level—at a probability of $P=0.05$. These observations thus do not exclude a teratogenic effect of the drugs but give no positive support for it.

Table IV presents a slightly different approach to

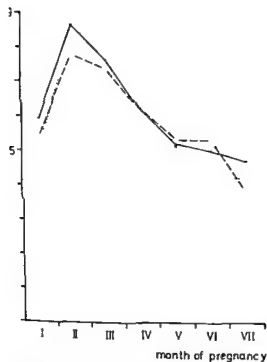


Fig 3 Percentage women using psychopharmaca during different months of pregnancy. Comparison between women who had non-malformed infants (—) infants with minor malformations (---) or infants with major malformations (-.-).

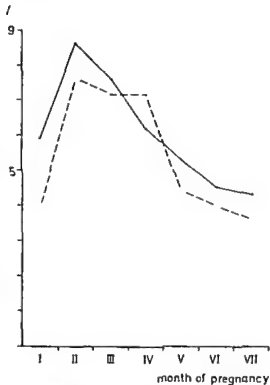


Fig 4 Percentage women using psychopharmaca during different months of pregnancy. Comparison between living normal infants (—) and the group of perinatal pathology (stillbirths or infants with major malformations) (---).

the material. 194 records of pregnancies that ended with the birth of an infant with a major malformation were compared with 194 matched controls. The latter were chosen from the first suitable questionnaire with the nearest consecutive number to each one in the study group. A similar series was collected of 93 infants who died before the age of one year but who had no malformation—53 of these were stillborn. Matched controls were selected as above. The first trimester use of psychopharmaca in general and meprobamate in particular is shown in the Table—the series has also been subdivided according to the occurrence of some different malformations. The expected number of users calculated from the total series (5 753 births) is also given.

In this series use of psychopharmaca occurred more often in the malformation group than in the control group ($P=0.05$). There were no differences between the group of dead infants and its control group. There are however lower incidences in all

Table III Malformations and stillbirths among infants to women who used psychopharmaca during pregnancy

Infant category	Total number	All psych pharm Trimester		Meprohamate	Barbiturate	Chlor diazepoxide	Hydroxyzine	Imipramine	Other anti depressive drugs
		1st	2nd-3rd						
All	5 753	815	456	263	372	89	16	13	16
Not malformed	5 007 (87.0%)	710 (81.9%)	390 (85.5%)	233	316	82	14	9	15
Minor malformation	551 (8.6%)	74 (8.5%)	49 (10.8%)	18	43	5	2	3	0
Insignificant major malform	105 (1.7%)	14 (1.6%)	9 (2.0%)	6	4	2	0	0	0
Gross major malform	95 (1.5%)	17 (2.0%)	8 (1.8%)	6	9	0	0	1	0
Stillbirth	68 (1.1%)	14 (1.6%)	3 (0.7%)	5	6	1	0	1	0

groups except that of malformed infants when comparison is made with the total series. The reason for this is not clear.

When the group of malformed individuals is broken down according to the malformations present, it is found that no major deviation occurs from expected numbers, with one possible exception: congenital heart defects. This may well be random, however. The slightly increased incidence at Mb Down would fit with the higher mean age at child birth for this group.

Pregnancy length and birth weight

Table V shows the distribution of length of pregnancy among normal infants born to women who used psychopharmaca during the first trimester or during the second and third but not the first trimester and those born to women who did not use psychopharmaca. There is a possible difference between the groups with a longer duration when the drugs had been used during the first trimester. This necessitates comparisons of birth weight being carried out at the same gestational age. Table VI shows

Table IV Number of women using psychopharmaca in general and meprobamate in particular species during the first trimester. Infants with major malformations and matched controls: infants without malformations but dead within one year with matched controls

Expected numbers calculated from the incidence of psychopharmaca users in the total material of women giving birth to non malformed infants (710/5 007) or from all controls

Infant group	Total number	No using psychopharmaca	Expected number from total material	No using meprobamate
Major malform	194	28	27.5	10
Controls to major malform	194	14	27.5	6
Dead but not malformed	93	9	13.2	3
Controls to dead but not malformed	93	9	13.2	5
Dead and/or major malform	287	37	40.7	13
All controls	287	23	40.7	11
<i>Specific malformation types</i>			<i>Expected no. from all controls</i>	
C.N.S. malform	11	2	0.9	2
Cleft lip and/or palate	10	3	0.8	0
Cong. heart malformation ± other malform	35	5	2.8	0
Hypoplasia	15	2	1.2	0
Cong. dysplasia of hip	37	1	3.0	0
Multiple malform	10	1	0.8	0
Mb. Down	10	3	0.8	2

Table V Duration of pregnancy when psychopharmaca were used during the first trimester during the second or third but not the first trimester and when such drugs were not used at all

Expected numbers (calculated for each sex separately) given within parentheses for pregnancies shorter or longer than 8-47 weeks

	Total number	Duration of pregnancy			χ^2 (4 d.f.)
		less than 38 weeks	38-47 weeks	more than 47 weeks	
boys					
drug first trimester	355	76 (77)	308	21 (15)	8.1 ()
drug 2nd-3rd trimester	186	18 (14)	166	2 (8)	
no drug	1 848	137 (140)	1 634	77 (77)	
girls					
drug first trimester	337	19 (77)	796	17 (11)	5.3 *
drug 2nd-3rd trimester	187	13 (17)	170	4 (6)	
no drug	1 859	129 (1 6)	1 673	56 (60)	

Between drug groups within sexes = 13.4 at 8 d.f.

S = non significant

The weight distribution among infants born within 2 weeks from expected date of birth (38-42 weeks of pregnancy). No effect of use of psychopharmaca on birth weight can be found

DISCUSSION

The use of psychopharmaca during pregnancy is obviously dependent on many different factors, some of which have been analysed in the present study. The desire for the pregnancy, the civil status

of the woman at LMP, the socio-economic class of the woman and her age are four factors which have been shown to affect drug use and these four factors are themselves obviously interacting. Some of them are known to be associated with pregnancy outcome, especially with respect to stillbirth rate (1).

Psychopharmaca are primarily used for mental diseases but are also prescribed for various other complaints. An unwanted pregnancy would be expected to favour the use of such drugs. This was also shown to be the case. As was demonstrated

Table VI Birth weights among non malformed infants born within 2 weeks before or after expected birth date (38-47 weeks pregnancy) when the woman had used psychopharmaca during the first trimester during the second or third but not the first trimester or had not used such drugs at all

	Total number	Birth weight, kg						χ^2 (10 d.f.)
		< 2.5	2.5-3	3-3.5	3.5-4	4-4.5	> 4.5	
boys								
drug first trimester	308	7	23	90	125	54	9	5.9 *
drug 2nd-3rd trimester	166	4	14	50	63	30	5	
no drug	1 634	8	147	563	600	250	51	
girls								
drug first trimester	796	7	41	13	89	18	9	18.2
drug 2nd-3rd trimester	170	7	70	64	51	5	3	
no drug	1 673	35	753	667	551	141	31	

Between drug groups within sexes = 4.1 at 10 d.f. N.S.

† 0.1 > P > 0.05

! = non significant

earlier (7) women who will later miscarry report a higher proportion of unwanted pregnancies than women who will later have a child and so an association between use of psychopharmaca and miscarriage would be expected and is also found. The incidence of drug use is however already higher among the miscarriage group of women during the first gestational month i.e. before the woman knows she is pregnant. Apparently other factors—probably of a social nature—exist which are associated with the risk of an unwanted pregnancy and which also favour psychopharmaca consumption.

Unwanted pregnancies are of course more common among women who are not married at the LMP than among women married at the LMP. Civil status and the desire for the pregnancy should therefore be expected to affect psychopharmaca consumption similarly. On the contrary women who are married at the LMP use more psychopharmaca during the first trimester than women unmarried at the LMP do. The effect of differences in desire for pregnancy must thus be balanced with other factors associated with civil status and leading to increased use of psychopharmaca. This is also illustrated in an analysis of the effect of socioeconomic class on psychopharmaca consumption. In such higher classes (Fig. 2) the rate of wanted pregnancies increases. As could be expected (7) this is paralleled by a decrease in smoking frequency—as smoking is correlated with unwanted pregnancy. On the other hand the use of psychopharmaca increases. From the calculations presented it is likely that both age of woman and another factor associated with civil status and therefore with socioeconomic class affect psychopharmaca consumption.

The increased use of psychopharmaca among women who will later miscarry is thus in effect of social factors and there are no indications that the use of such drugs in itself increases the risk of miscarriage—a phenomenon exactly paralleling what was found for smoking (7). The late rise in psychopharmaca consumption noted for women who will have a late spontaneous abortion may perhaps be due to medication caused by signs and symptoms indicating the threatening abortion. It is however also interesting to note that the increased smoking among women who will have a miscarriage was mainly restricted to those who will have a late abortion (7). There are no indications of an association

between use of psychopharmaca and the birth of a dead or defective child. Richards (17) described an association between the birth of a child with a major malformation and the use of sedatives during the first trimester. Crombie et al. (3) also found an excess of barbiturate prescriptions among women who will have a malformed child compared with controls and the same finding was reported by Nelson & Forfar (11). Villumsen (14) could not record any effect of barbiturate or other psychopharmaca on malformation rate. Belafsky et al. (1) compared pregnancy outcome in 900 women who used meprobamate during pregnancy with 39 other gravidas from the same region without finding any adverse effects. The incidences of deformities recorded in both groups were so low however that ascertainment of developmental defects must have been inefficient. A lower abortion rate was described in the meprobamate group than in the control group—a finding in variance with ours.

No firm evidence for a teratogenic effect was found in the present investigation. The use of psychopharmaca is equally common in the group of women who will have an infant with some sort of malformation as in the control group of women who will have a normal infant and no differences could be found between minor or major malformations. When women who gave birth to infants with major malformations were compared with matched controls a difference just reaching statistical significance was obtained with a higher incidence of drug use in the study group than in the control group. The study group had an incidence however which agreed well with the overall incidence recorded in the total series. Either the lower drug use in the control group was a random phenomenon—but it is perhaps made less likely by the fact that other further groups showed the same low incidence—or the matching process resulted in a selection of non-users. It is hard to understand how the matching process would have occurred but seasonal use is one factor which can play a role as it is well known that some malformations show a seasonal pattern in incidence. The difficulty in identifying any specific drug or any specific malformation in the association tells against a true association between drug use and teratogenesis.

In experimental teratology a drug can affect the malformation rate and fetal growth. An effect was therefore made to compare birth weight in infants born to women who used psychopharmaca

infants born to women who had not. It was found that pregnancy length may differ—there was a shift towards longer pregnancies when the woman had used such drugs during the first trimester. If this is not a random finding, it could be a drug effect. Certain drugs—so far mostly anti-inflammatory drugs have been discussed from this point of view—can inhibit prostaglandin synthesis and release.

13) The use of such drugs during pregnancy could through this mechanism prolong gestation time.

10) A sedative effect on hypothalamic centres and the posterior pituitary could also be a possible explanation for a drug effect. Social factors which have been shown to be of importance for drug usage and social class could perhaps also be of importance in this respect.

When birth weights were studied within one group—non malformed infants born at term (38–42 weeks pregnancy)—no effect of psychopharmacase on infant weight was found.

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DISC ELECTROPHORETIC PROTEIN PATTERN OF CERVICAL MUCUS IN CASES OF HUMORAL SENSITIZATION AGAINST SPERMATOZOA

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Abstract. Midcycle cervical mucus samples from 70 fertile women with negative and from 6 infertile women with positive serum sperm agglutinating activity were subjected to a qualitative protein analysis by the Ouchterlony technique to polyacrylamide (PAA) disc electrophoresis and after purification on spermatozoa they were also run against rabbit antihuman serum in the aureli immunoelectrophoretic technique. By the Ouchterlony method the main serum protein fractions were shown to occur also in cervical mucus. No significant differences could be found in the protein pattern of samples from fertile and infertile females as detected by polyacrylamide (PAA) electrophoresis. In all samples a protein fraction corresponding to serum albumin and a band preceding the protein fraction corresponding to serum prealbumin were observed. In 2 samples from infertile females it could be demonstrated that immunoglobulins occurring in the cervical mucus were directed towards antigenic sperm components.

There has been quite a lot of recent comment about the protein fractions secreted along with local body fluids (8-9). The protein components of female genital tract secretions are thought to be important in connection with fertility. The protein fractions which occur in the cervical mucus of normal individuals have been widely discussed by Moghissi & Kuhns (10), Schumacher (14) and Odeblad (11). No clear information is available about the protein composition of cervical mucus with respect to fertility.

In the present paper the protein pattern of cervical mucus in selected cases of infertile women with strong humoral antisperm activity are described.

MATERIAL AND METHODS

Cervical mucus samples from 6 infertile women presenting agglutinating humoral antibodies against spermatozoa,

determined by the Franklin & Dukes method using 1:4 serum dilutions and of 10 fertile women without humoral sperm agglutinating activity were collected at midcycle under identical conditions by mild suction with a syringe connected to a conical polyester tube. The samples were stored at -70°C and after thawing subjected to the following procedure.

1 All samples were subjected to discontinuous polyacrylamide (PAA) electrophoresis (Ornstein 1964; Davis 1964) using a 3.1% stacking gel and a 7.1% separating gel in Tris glycine buffer, pH 9.0. Vertical columns of 4.7 mm inner diameter, 60 mm length, 40 mm running gel and a current of 5 mA per column were used. The protein content was adjusted individually to a total applied protein content of 250-350 µg per column.

2 The occurrence of serum proteins in the cervical mucus was detected by the qualitative immunoprecipitation method after Ouchterlony (1964).

3 For the specification of immunoglobulins identified with the Ouchterlony technique the cervical mucus samples from fertile women and women with humoral agglutinating activity against spermatozoa were purified on spermatozoa by affinity chromatography in a batch device and their antibody content and type determined with rabbit antihuman serum in the two-dimensional immunoelectrophoresis (7).

RESULTS

The PAA pattern of cervical mucus from normal fertile women compared with the corresponding protein pattern in serum is demonstrated in Fig. 1. In addition to the protein fractions electrophoretically corresponding to the gamma globulins and haptoglobulins of the sera, a protein band regularly occurs in cervical mucus which corresponds to transferrin-C of human serum. In the typical locus of serum albumin, two fractions of high density are detected in PAA electrophorographs of cervical mucus. This pattern of the albumin with two major

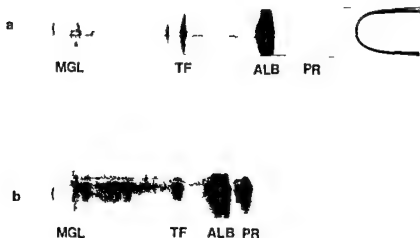


Fig 1 Comparison of PAA pattern of serum (top) and cervical mucus (bottom) of a normal fertile woman at midcycle

peaks was found only in 6 out of the 20 normal mucus samples examined. In all these 6 cases the serum albumin fractions presented a single albumin peak (Fig. 1). The most rapidly migrating protein fraction corresponds electrophoretically to the serum prealbumin fraction which occurred in all cervical mucus samples investigated. In contrast to the serum prealbumin, this band revealed considerable intensity in gels stained with Coomassie blue. Furthermore, in 4 cases an additional band occurred before the band corresponding to the serum prealbumin fraction which resembled serum urosomucoid.

In 6 cases of cervical mucus from infertile women presenting a positive humoral sperm agglutinating activity the basic protein pattern of a normal mucus sample was detectable. In 7 cases considerable enlargement of the macroglobulin fraction could be found (Fig. 2). This may be related to an artefact due to a poor mucus solubility. A specific gammaglobulin fraction cannot be detected by this particular technique.

The Ouchterlony technique used revealed the occurrence of the following protein fractions in cervical mucus from normal and infertile women: prealbumin, albumin, alpha acid, glycoprotein.

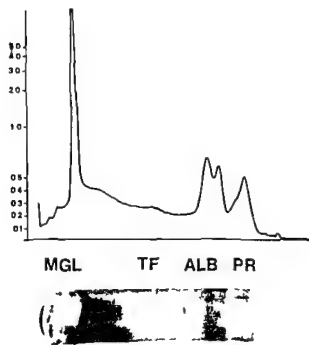


Fig 2 Densitograms of electrophoretic protein pattern of cervical mucus of 2 infertile women presenting $46 \pm 6\%$ and 28% sperm agglutinating serum activity.

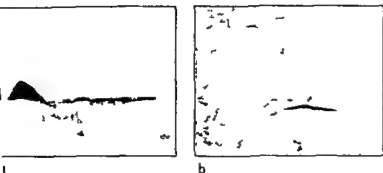


FIG. 3 Immunoelectrophoresis of an affinity chromatography purified cervical mucus sample from an infertile woman with sperm agglutinating serum activity (a) run against rabbit antihuman serum in comparison with a normal mucus sample (b).

transferrin, haptoglobulin, immunoglobulins, IgG and IgA. No immunoglobulins IgM or α_2 macroglobulin or ceruloplasmin could be verified.

Applying crossed immunoelectrophoresis only in the 2 cases of sperm agglutinating serum activity a specific antiserumal cervical mucus immunoglobulin was defined by the typical localization of the corresponding precipitation band as well as by absorption studies could be demonstrated (Fig. 3). In these 2 cases the antibody could be defined as IgA while serum agglutinating antibodies could be identified as IgM.

DISCUSSION

The results presented show a similar protein pattern of cervical mucus and serum. Major differences result from the occurrence of a split albumin fraction and the occurrence of a protein fraction preceding the band corresponding to serum prealbumin. To our knowledge neither phenomenon has been described previously. No major differences could be detected between the protein pattern of the mucus samples from fertile and infertile women except for the cases of infertile females. In these 2 cases a considerable increase in the protein fractions corresponding to the serum gamma globulin region could be observed in the cervical mucus.

In these 2 cases, however, do not permit any reliable conclusions. Protein analysis of cervical mucus has been accomplished by numerous authors using different separating techniques. Disc electrophoretic studies performed by Beier & Beier (9) revealed a similar pattern as presented in this report. The authors, however, do not relate their results to infertility.

The desired aim of cervical mucus protein analysis in cases of female infertility due to or

associated with serum sperm agglutinating activity has been to disclose an accumulation of antiserumal factors perhaps immunoglobulins in the local genital secretion.

Immunoglobulins, however, proved to occur almost constantly in cervical mucus (9).

Furthermore, even major variation in immunoglobulin content of the cervical mucus would hardly give rise to a detectable alteration in electrophoretic patterns. Moreover, as to PAA media, some Ig's have not always been found to migrate in the PAA gel concentrations commonly used. In addition, it has not yet been demonstrated that immunoglobulins occurring in cervical mucus of women with serum sperm agglutinating or immobilizing activity are actually directed toward antigenic sperm component. The last step of our studies attempted to clarify this question. Sperm specific immunoglobulin purified by affinity chromatography on spermatozoa in a batch device migrating into a rabbit antihuman serum showed one precipitation line that could be identified by localization and absorption as IgA.

To our knowledge, this is the first evidence in favour of the idea that immunoglobulins found in cervical mucus are directed against sperm antigens. This is supported by the observation that such a positive reaction could not be found in the mucus of women without humoral sperm antibody activity when subjected to the same procedure (Fig. 3).

The results make it possible to measure the antiserumal portion of immunoglobulin occurring in cervical mucus. Schulmann (15) in 19 mucus samples from women with sperm agglutinating serum activity found 11 with a positive sperm agglutinating reaction using the Kibrick test and 10 by using Franklin & Dukes test.

These results accord well with our unpublished data on the occurrence of about a ten times lower sperm agglutinating activity in cervical mucus samples as compared with the serum. The application of sensitive protein analytical methods as the Laurell immunoelectrophoresis and affinity chromatography (5) reveal effective antisermin activities of cervical mucus, uterine and tubal secretions undetectable with the current low sensitive serological methods.

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STUDY OF THE SUPPRESSION OF LACTATION AND THE INFLUENCE ON BLOOD CLOTTING WITH BROMOCRIPTINE (CB 154) (PARLODEL®) A DOUBLE BLIND COMPARISON WITH DIETHYLSTILBOESTROL

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Abstract. Inhibition of lactation was studied in 38 puerperal women in a double blind trial to assess the effect of bromocriptine in comparison with diethylstilboestrol (DS). Simultaneously the influence of both compounds on blood clotting was studied along with a control group of 30 women not receiving any medication. Bromocriptine was given in a daily dose of 5 mg for 14 days and DS in a daily dose of 0.1 mg for 7 days followed by a placebo for a further 7 days. The first doses were not later than 8 hours after delivery. Both compounds showed an inhibitory effect on the onset of lactation and mammary congestion. This inhibitory effect on both parameters was significantly in favour of bromocriptine during the last days of the treatment due to rebound in the DS group. The bromocriptine doses used (5 mg daily for 2 weeks) caused no objective side effects and no subjective restraint. Treatment with bromocriptine caused no untoward effect on the blood clotting, while in the DS group a slower turn to normal antithrombin III could be observed. Also in this group one case of thrombophlebitis occurred. Bromocriptine can be administered to puerperal women for the suppression of lactation.

Only a small choice of therapeutic measures were available until recently for the suppression of lactation in puerperal women.

Most commonly used is hormonal therapy with oestrogens and androgens in order to prevent pain and engorgement of the breasts. However, there is a finite risk of thromboembolic complications (12-15). The need for a potent and well tolerated drug to suppress lactation is therefore evident.

Bromocriptine is the polypeptide ergot alkaloid 2- α -Br- α -ergocryptine. The compound has a specific inhibitory effect on prolactin secretion both directly at the hypophyseal level by blocking pro-

lactin release from the hormone producing cells (8, 20-26) as well as indirectly by increase of hypothalamic prolactin inhibiting factor activity (32).

The antigalactic effect of bromocriptine has proved successful in several animal studies (2, 9, 10, 25) and recently in puerperal women (4, 23, 24, 29, 31). The compound is also active in patients with galactorrhoea/amenorrhoea syndrome (5, 7, 14, 16, 17, 21, 22, 28) as well as in male patients with galactorrhoea and impotence (1).

This report concerns the effect of bromocriptine and diethylstilboestrol (DS) on the onset of puerperal lactation and the blood clotting factors in a double blind trial.

MATERIAL AND METHODS

Thirty-eight puerperal women entered this double blind comparative trial. In addition to this, a control group of 30 puerperal women was formed in order to obtain comparative values for blood clotting factors. 30 women received CB 154 and 18 women were given DS. Women with severe metabolic disturbances or with concomitant therapy, e.g. corticoid, thyroid, anti-thyroid therapy, diuretics, phenothiazines, which might influence results of the study, were excluded from the outset.

There was no statistically significant difference between the mean values for the two groups as to age, body height, body weight, parity, duration of pregnancy, duration of labour, type of delivery (spontaneous/operative), systolic and diastolic blood pressure, pulse rate as well as to the pretreatment values of the blood clotting tests (Table I).

The oral medication schedule was as follows:

1. CB 154 capsules containing 2.5 mg of active compound, one capsule orally in the morning and in the even-

Table I Basic data

	CB 154 group	DS group	Test method	P
<i>Age</i>				
Mean value (years)	30.0	27.6	U Test	0.59 n
Extreme values	17-36	19-45		
<i>Body height</i>				
Mean value (cm)	151.1	167.1	U Test	0.37 n
Extreme values	156-178	167-178		
<i>Body weight</i>				
Mean value (kg)	72.8	74.5	U Test	0.43 n
Extreme values	58.2-94	61-88.5		
<i>Number of pregnancies</i>				
0	4	5	2I Statistics	0.86 n
1	5	3		
2	8	5		
3	1	2		
4	1	2		
5	1	1		
Mean value	1.7	1.8		
<i>Duration of pregnancy (days)</i>				
Mean value	258.1	259.8	U Test	0.83 n
Extreme values	210-290	210-280		
<i>Duration of labour</i>				
Mean value (hours)	7.4	6.7	U Test	0.54 n
Extreme values	0.5-27.5	1-45		
<i>Type of delivery</i>				
Spontaneous	18	16	2I Statistics	0.95 n
Perforation of skull (cranio-lasis)	1	1		
Forceps	0	1		
Caesarean section	1	0		
<i>Intervall between delivery—1st drug intake</i>				
Mean value (hours)	4.5	4.4	U Test	0.99 n
Extreme values	1-8	0.8-8		

ing before meals during 14 consecutive days for a total of 28 capsules (70 mg).

2 DS capsules containing 10 mg of active compound one capsule orally in the morning and in the evening before meals during 7 consecutive days for a total of 14 capsules (140 mg) followed by further 7 days of placebo treatment administered in the same way.

The 7 day administration of DS complies with the most commonly used medication schedule which is considered to be relatively safe regarding thromboembolic complications.

All capsules were identical in appearance. The allocation of the patients to the two treatment groups was done with the help of a randomisation list. The first dose of drug was given not later than 8 hours after delivery.

The patients were examined daily during their stay in hospital by both doctor and midwife. Examinations by doctor were performed on each patient at the beginning and on day 14 of the treatment. The following effects were assessed and scored: Mammary secretion, mammary con-

Table II Scoring system for determination of mammary secretion, mammary congestion, occurrence of side effects, involution of uterus and overall assessment

Score	Mammary secretion (by palpation)	Mammary congestion	Side-effects
0	No milk	Absent	Absent
1	Some drops	Slight	Slight
2	Slight outflow	Moderate	Moderate
3	Stream of milk	Severe	Severe
	Involution of uterus	Overall assessment	
0		No effect	
1	Poor	Poor	
2	Good	Good	

Table III Prevention of mammary secretion

group		Treatment day				
		1st	6th	12th	13th	14th
CB 154	Secretion/Total no of patients	0/20	3/20	1/20	1/20	2/20 ^{a)}
DS	Secretion/Total no of patients	1/18	0/18	8/18	7/18	6/18
)		$P < 0.1$	$P < 0.05$	$P < 0.01$		

stion involution of uterus occurrence of side effects
 general assessment by doctor midwife and patient The
 oning are shown in Table II

The side effects which were recorded were either
 observed changes which were uncommon in puerperal
 women or subjective complaints spontaneously men-
 tioned by the patients when they were questioned about
 unusual feelings. However no leading questions for
 specific symptoms were asked.

Systolic and diastolic blood pressure and pulse rate
 were recorded during the stay in hospital and on the last
 day of the treatment.

Haemoglobin levels erythrocyte and leucocyte counts
 renal and hepatic function laboratory tests were also
 performed.

A possible influence on blood clotting was assessed in
 three groups by the following tests:

Thrombocyte count (3) Normotest (19) Ethanol gela-
 tin test indicating fibrinemia (17) Citrated plasma was
 stored at -20°C until immunoassay of antithrombin III
 (At III) α_2 macroglobulin ($\alpha_2\text{M}$) fibrinogen and
 plasminogen.

Immunoassays were performed with the Mancini tech-
 nique (18). Rabbit antiserum against At III was obtained
 from Nyegaard & Co. Oslo, Norway. The other antisera
 were obtained from Behringwerke Marburg/Lahn-
 stein. Antifibrinogen serum was used for assay of
 DP. In order to avoid coagulation the gel buffers
 contained EDTA 1 g/1000 ml (10).

After conclusion of the treatment the follow up was
 aided with the help of a simple questionnaire which was
 sent back to the investigator after the first post partum
 menstruation. Milk secretion as continuing lactation or a
 bound phenomenon spotting and/or the recommence-
 ment of menstruation were recorded on the basis of pa-
 tients' replies.

Statistical analyses concerning homogeneity of both
 groups were done by χ^2 Statistics or by U Test respec-
 tively. Mammary secretion mammary congestion uterine in-
 volution general assessment coagulation test values be-
 fore treatment spotting and rebound phenomena were
 evaluated by χ^2 Statistics only. Blood pressure and pulse
 rate were analysed by both Wilcoxon Test and U Test.
 The comparison between both groups with regard to dura-
 tion of post partum amenorrhoea was performed by
 U Test.

RESULTS AND COMMENTS

Prevention of mammary secretion (Table III)

Both preparations have shown an inhibitory effect
 on the onset of milk secretion. During the last few
 days of the treatment there was a statistically
 significant difference in favour of CB 154 ($P < 0.01$)
 on the 13th day) due to rebound in the DS group.
 During the 10th to the 14th day of the study a
 postponed onset or continuation of lactation was
 observed in 8 of the 18 DS treated women. In con-
 trast to these findings milk secretion was seen in
 only 2 of the 20 CB 154 treated women during this
 period. This difference is statistically significant on
 days 12 ($P < 0.05$) and 13 ($P < 0.01$) in favour of CB
 154.

Mammary congestion (Table IV)

This symptom occurred more frequently in the DS
 group than in the CB 154 group ($P < 0.05$) due to
 rebound in the DS group. Artificial emptying of the
 breasts was not necessary in any of the women.

Table IV Occurrence of mammary congestion

group		Treatment day		
		1st	7th	14th
CB 154	Congestion/Total no of patients	0/20	1/20 n.s.	0/20
DS	Congestion/Total no of patients	1/18	1/18	4/18
)		$P < 0.1$	$P < 0.05$	$P < 0.01$
			$P < 0.01$	$P < 0.001$

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LEUCINE AMINOPEPTIDASE ACTIVITY IN MATERNAL CORD BLOOD AND PLACENTA OF NORMAL PREGNANCY AND IN PRE ECLAMPSIA

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Abstract 1) Leucine aminopeptidase (LAP) was determined in maternal and fetal blood and in the placenta of mild and severe pre-eclamptic patients then compared to the levels of normal pregnancy 2) The enzyme activity increased significantly in severe pre eclampsia as compared with the mild type and with normal pregnancy 3) In mild pre-eclamptic patients LAP activity of maternal and cord blood did not increase significantly as compared with the normal cases but the placenta showed a slight decrease 4) The enzyme level in cord blood did not increase markedly in pre eclampsia

Leucine aminopeptidase (LAP) is a proteolytic enzyme needed in the terminal digestion of L leucyl peptides or the transfer of L leucine from one peptide mole to another. It occurs principally in the small intestinal mucosa and in pancreatic extracts although it has been found in most human tissues, animals, plants and microorganisms. The function of this enzyme in the blood is not known.

Previous studies had clearly demonstrated that serum LAP activity increased during pregnancy (1-9) and that the serum level of this enzyme returns to normal 6-8 weeks post partum. (1) reported increased serum LAP activity in normal pregnancy reaching the highest level during the last trimester and specifically at the time of delivery although the enzyme level in the cord blood was within normal limits.

The substrate used for measuring LAP activity is hydrolysed by oxytocinase (3, 10) and this accounts for the increased LAP activity in the serum of pregnant women. It was shown that serum oxytocinase tends to decrease in pre eclampsia (6). (2)

also demonstrated that decreasing levels of serum oxytocinase in severe toxemia indicated diminishing placental function and impending fetal death. On the other hand low level serum oxytocinase was demonstrated in patients with placental insufficiency (8) possibly due to impaired production or an excessive urinary leak of this enzyme. The latter author also found that severe toxemia was accompanied by a marked increase in the urinary excretion of leucine aminopeptidase although the urinary excretion of this enzyme remained relatively low throughout normal pregnancy as compared to normal non pregnant women.

The purpose of this study is to measure the leucine aminopeptidase levels in maternal serum, placenta and cord blood in pre eclamptic patients as compared with normal pregnancy and to assess its diagnostic value as a measure of placental function.

MATERIAL AND METHODS

Thirty pregnant women were selected from the Maternity department of Ain Shams University Hospitals and they were classified into three groups (Table I): (a) 10 cases of normal uncomplicated pregnancy; (b) 10 cases of mild pre-eclampsia; (c) 10 cases of severe pre eclampsia.

In this classification of mild and severe pre-eclampsia we followed the international standards of the Organisation Gestosis (7).

We collected the samples of maternal blood before the onset of labour while the placental and cord blood were taken immediately after labour. All deliveries were vaginal with no complications and anaesthesia if needed was either local infiltration or pudendal block anaesthesia.

Table I *The description of 10 normal pregnancies and 20 pre eclamptic patients submitted for enzyme estimations of the maternal and cord blood and placental tissues*

Type of cases	Age (y)	Parity	Gestosis index (G I)*
Normal pregnancy			
Mean	26.9	2.6	0.27
S.D. ±	5.7	2.8	1.03
Mild pre eclampsia			
Mean	27.1	2.30	3.80
S.D. ±	5.6	2.02	0.79
Severe pre eclampsia			
Mean	29.6	1.40	8.87
S.D. ±	8.99	2.17	1.85

G I is calculated by the sum of ratings of the edema, proteinuria, systolic and diastolic blood pressures.

Blood samples were allowed to clot and the serum separated was kept at 4°C for the enzyme assay within 24 hours.

Placental samples were taken according to Ramadan et al (7) washed with ice cold distilled water then homogenized in distilled water for three minutes at a concentration of 1 mg/ml. The homogenates were centrifuged at 2000 r.p.m. for 5 minutes and the supernatant fluid was used for LAP assay.

Aliquots of the maternal and cord blood sera and the supernatant fluid from the placental homogenate were used in duplicate for assaying LAP activity depending on hydrolysis of the substrate L-leucyl B-naphthylamine hydrochloride where B-naphthylamine will be freed and converted to a coloured azo dye by means of a diazotization reaction. The colour density of the azo was assayed

spectro-photometrically at 560 mμ according to method of Goldberg, Pineda and Rutenburg (4). Results were expressed in micrograms of B-naphthylamine liberated by 1 ml of a 2% dilution of the serum which is enzyme unit.

RESULTS

Leucine aminopeptidase (LAP) activity showed significant increase in maternal and cord blood sera and the placenta of severe pre-eclamptic cases compared with the corresponding values of mild pre-eclamptic patients and with normal pregnant women.

The enzyme activity in mild pre-eclamptic patients indicated a statistically not significant increase in the maternal and cord blood sera as compared with normal pregnant women but the placental values from the first group were somewhat higher than normal cases.

The statistical analysis of LAP activity was carried out using Student's *t* test corresponding *P* value to indicate the difference between each mild or severe pre-eclamptic and normal pregnant women for maternal and cord blood sera and for the placenta. All data are presented in Table II.

DISCUSSION

It has been reported that leucine aminopeptidase enzyme is one of the changeable enzymes during pregnancy. The general object of this study was to see if any particular pattern of change is used.

Table II *The estimation of leucine aminopeptidase enzyme in normal pregnancy and in pre-eclampsia*

Leucine aminopeptidase (LAP)	Maternal blood ^a			Cord blood ^a			Placenta ^a		
	Pre-eclampsia		Normal preg	Pre-eclampsia		Normal preg	Pre-eclampsia		Normal preg
	Mild	Severe		Mild	Severe		Mild	Severe	
No. of cases	10	10	10	10	10	10	10	10	10
Range	24-55	40-60	75-200	12-21	17-20	19-26	35-65	35-55	60-100
Mean	42.0	49.0	107.0	17.1	18.5	21.8	51.0	49.2	61.0
S.D. ±	8.84	6.85	35.52	3.0	1.048	2.49	8.14	5.85	6.1
Test of significance (P)	N.S.			N.S.			N.S.		
	<i>P</i> < 0.001			<i>P</i> < 0.002			<i>P</i> < 0.001		
	<i>P</i> < 0.001			<i>P</i> < 0.002			<i>P</i> < 0.002		

LAP in terms of μg B-naphthylamine/ml

* LAP in terms of μg B-naphthylamine/mg fresh tissue

the diagnosis of placental function in abnormal pregnancy. Our results indicated an elevated level of enzyme activity in the sera of normal pregnant women before the onset of labour (mean 42 units) as compared with 10.8 units recorded by previous authors as Mullan (8) for non pregnant women whereas LAP activity in the sera of the cord blood of the same cases was very low. The enzyme activity in the placenta of normal pregnancy was distinctly higher than that of the corresponding serum. The hydrolysis of the substrate used for LAP activity by oxytocinase activity (3-10) may partially account for the increased results in normal pregnancy but this should not be taken into consideration in placental insufficiency and in pre-eclamptic cases where the oxytocinase activity decreased in serum (2.6-9). Accordingly the results obtained in pre-eclamptic patients mainly represent the LAP activity. In severe pre-eclampsia LAP activity had significantly increased in serum, placenta and cord blood as compared with normal pregnancy and this confirms the marked increase in its urinary excretion recorded by Mullan (9). Its high serum levels due to its spilling over in urine. The elevation of LAP activity in mild pre-eclamptic cases in serum and cord blood was not significant as compared with the normal group but the placenta showed a marked decrease. A sharp increase in LAP activity of statistical significance was observed in maternal blood, cord blood and placenta on comparing severe with mild pre-eclamptic cases. The highly significant increase of LAP activity in maternal blood and placenta of severe pre-eclamptic patients may be due to the degeneration and infarction of the placenta with release of the enzyme in the maternal circulation. Thus the placenta can be considered as the main source of LAP enzyme in the maternal blood. The fetal blood level of LAP enzyme was not greatly affected in pre-eclampsia indicating that the enzyme may not cross the placental barrier.

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ON RADIATION DECREASED FIBRINOLYTIC ACTIVITY OF VESSEL WALLS

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Abstract Fibrinolytic activity was histochemically determined in biopsy specimens of the walls of human veins (epigastricae superficiales) obtained from 15 patients after irradiation of the lower abdomen because of malignant diseases and compared with non-irradiated controls. The activity was significantly lower ($p < 0.001$) after radiotherapy.

The endothelium of certain vessels, especially veins, contains activators of fibrinolysis which are liberated into the blood stream (6). These activators are important for counteracting thrombosis (4) and in the repair (1). They have also been studied in the walls of ovarian tumour vessels and have been considered to be essential for growth of such lesions (5).

Malignant diseases treated with radiation are attended by an increased frequency of thrombosis which often starts in irradiated regions (8). Irradiation also alters tissue repair (9). Irradiation of a tumour damages its vessels (11) and might help to inhibit its growth—These effects of radiation may be partly explained by damage to the vessel endothelial cells responsible for the production of fibrinolytic activator enzymes.

To check the validity of this assumption we determined the histochemically determined fibrinolytic activity in biopsy specimens of the walls of human vessels in patients after radiotherapy and in non-irradiated controls.

MATERIAL AND METHODS

Clinical material consisted of 15 patients who had received radiation treatment of the lower abdomen because of carcinoma of the uterine body or cervix, stages I-III.

Venous biopsy specimens about 1 cm long were obtained from the left and the right superficial epigastric vein at operation via a Pfannenstiel incision. The patients had received a total dose of 2 000-4 300 rad in this area.

The interval between the end of radiotherapy and the operation was 5-13 weeks in 13 and 2½ years in 2. 14 patients of comparable age who had been operated upon because of non-malignant conditions and who had not received medicinal or hormone therapy served as controls.

The biopsy specimens were quickly frozen in rapidly expanding CO₂ to prevent freezing artefacts. They were packed hermetically in Parafilm® to prevent dehydration and were stored at -60°C until examined.

The fibrinolytic activity was measured with the histochemical method of Todd as modified and graded by Pandolfi et al. (7). The vessels were cut on an International Harris cryostat in sections 8 µ thick and collected on pre-cleaned glass slides. Four slides were prepared for each sample. The sections on each slide were covered with 0.06 ml of fibrinogen (bovine fibrinogen essentially prepared according to Brakman (8)) in concentration of 1% in phosphate buffer, pH 7.8, ionic strength 0.15 and of 10 µl thrombin (Topostasin 70 NIH units/ml unbuffered saline).

The fibrinogen-thrombin mixture was spread over an area of 10 mm² in order to obtain a fibrin film about 0.07 mm thick. The slides were left at room temperature (21-4°C) in a moist chamber for 30 minutes to stabilize the fibrin film. One of the slides was immediately afterwards fixed in formalin while the remaining 3 slides were transferred to another moist chamber at 37°C and incubated for 10, 20, 30 minutes respectively after which they were fixed in formalin. The fibrin slides and sections were stained with Harris haematoxylin. Fibrinolysis was reflected by clear lytic areas in the fibrin film at the site of fibrinolytically active cells. Three fairly distinct grades of fibrin digestion were recognized: *grade I* microscopical punctate areas in most of the sections; *grade II* gross lytic areas of irregular outline and sometimes confluent; *grade III* dissolution of most or all the fibrin in contact with the sections. A grade I slide was allotted 1 point, a grade II slide 2 points and a grade III slide 3 points. The total number of points scored by the set of 4 slides was taken as a measure of the fibrinolytic activity of the sample.

Table 1 Fibrinolytic activity of walls of irradiated and non irradiated superficial veins (median value and range in arbitrary units)

Vein	Non irradiated	Irradiated
Superficial epigastric vein		
Left	8 (6-9.5)	3 (0.5-5.5) **
Right	8.25 (5.5-9.5)	3.5 (1.5-6)***

*** $p < 0.001$

Comparisons between the fibrinolytic activity of different groups of veins were made by the Wilcoxon rank sum test (3)

RESULTS

The fibrinolytic activity of the specimens from the irradiated vessels was significantly lower ($p < 0.001$) than in that of the controls (Table 1). There was no significant correlation between the decrease in the fibrinolytic activity and the doses given. But there was a tendency of the fibrinolytic activity to decrease with increasing interval between radiation and sampling (Fig. 1). The two patients operated upon 2½ years after radiation still had low values (2 and 3.5 arbitrary units).

DISCUSSION

The results thus show that the fibrinolytic activity from irradiated vessels is significantly decreased compared with that of the controls. We know from earlier observations in our laboratory that there is no significant difference in the fibrinolytic activity of

veins (superficial epigastric veins) from patients with cancer compared with those of healthy volunteers (unpublished data).

In tissue culture studies it has been shown that fibrinolytic activators are synthesized in the vessel wall probably in the endothelium (13). Irradiation directly damages the endothelial cells (11) it might also affect the nuclear DNA synthesis and effects the cytoplasm mainly the structures which synthesize enzymes (5). This helps to explain our finding of significant reduction in the fibrinolytic activator content of vessel walls after radiotherapy. We did not find the reduction of the fibrinolytic activity to correspond with the doses given. There seems to be a connection however between the reduction of the fibrinolytic activity and the interval between radiation and sampling (Fig. 1). This is in agreement with observations in animal experiments by Laks et al. (12) and with the present finding of still low values in the patients examined 2½ years after radiation which suggests a permanent inhibition of the synthesis of the activators. The results may help to explain such things as increased frequency of thrombosis, change in tissue repair and inhibition of tumour growth in irradiated patients.

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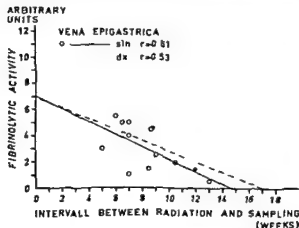


Fig. 1 Comparison between fibrinolytic activity in superficial veins and interval between radiation and sampling.

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GROWTH OF THE FETAL SKULL WITH SPECIAL REFERENCE TO WEIGHT FOR DATES OF THE NEWBORN CHILD

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Abstract Two growth curves of the fetal biparietal diameter are presented. One is prospective based on the last menstrual period while the other is retrospective calculated from the date of birth. The prospective curve shows slight decline in average values following the week 42 of station. This is thought not to reflect a true shortening of the biparietal diameter in the single fetus since serial measurements in single fetuses show growth up to the time of delivery. Individual growth curves grouped according to the weight for-dates of the newborn child show trend towards longer biparietal diameters in the larger fetuses. Serial measurements preferably starting before week 30 appear to be a good help in predicting birth weight but growth rates without regard to the actual measurement values were of little prognostic value in the present material.

Several curves of the normal growth of the fetal skull measured by pulsed ultrasound have been presented (4, 5, 6, 8). These are similar in shape but they show parallel shifts along the metric axis. These differences are probably due to different apparatus calibration. Single measurements have been valuable in the assessment of fetal age (5). Serial measurements have given more accurate information about fetal prognosis than urinary estriol assays (7). A previous report from our department showed a correlation between the fetal biparietal diameter and the weight for-dates of the newborn child in a small series of pregnancies complicated by pre-eclampsia (1). We have now extended our observations and will present normal curves for the biparietal diameter based both on prospective and retrospective pregnancy dating. Furthermore in this new series we will report the results of all individual cases of normal and pre-eclamptic pregnancies in which two or more measurements were performed clas-

sified according to the weight for-dates of the newborn child.

MATERIAL AND METHODS

Normal pregnancies These were defined as single pregnancies in women with no pregnancy complications and live children without malformations. These form the basis of the normal curves. The material consists of 165 women with normal pregnancies.

The prospective normal curve This was based on a definite dating of the last menstrual period in women with regular periods who did not take contraceptive pills shortly before the actual pregnancy irrespective of whether the pregnancy was terminated by induction or spontaneously. Altogether 131 pregnancies fulfilled these criteria.

The retrospective normal curve This was constructed retrospectively from the time of delivery in women who went into labour spontaneously. Altogether 54 pregnancies fulfilled these criteria.

Classification of the fetuses The fetuses were classified

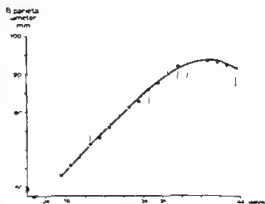


Fig. 1 The smoothed normal prospective curve. The actual figures are given in Table I.

Table I Weekly average values of the biparietal diameter in cases of normal pregnancy based on definite dating of the last menstrual period

Gestational age (weeks)	No of observations	Mean (mm)	S D
24.5	3	70.7	5.5
25.5	1	63.0	0.0
26.5	5	65.6	5.3
27.5	13	71.6	5.4
28.5	13	71.3	6.2
29.5	10	73.0	3.1
30.5	21	75.7	4.7
31.5	20	77.4	6.2
32.5	19	81.1	5.5
33.5	26	82.7	4.5
34.5	25	85.6	4.8
35.5	36	87.5	3.0
36.5	32	88.6	4.1
37.5	46	91.8	8.1
38.5	45	91.0	3.5
39.5	50	92.3	3.4
40.5	29	93.0	3.8
41.5	17	92.9	3.2
42.5	6	92.2	2.6
43.5	4	90.0	2.9

retrospectively in degrees of maturity according to standard tables of birth weight and gestational age (3). These tables were based on 175 485 births in Norway 1967 and 1968

- large for dates birth weight above 90th percentile
- medium-sized upper range birth weight between 50th-90th percentiles
- medium-sized lower range birth weight between 10th-50th percentiles
- small for dates birth weight below 10th percentile

The material comprises 22 children in group *a*, 66 children in group *b*, 88 children in group *c*, and 16 children in group *d*.

Measurements The biparietal diameter was measured by pulsed ultrasound using a diagnostic A and B scan unit made by Kretz Technik (series 4100 MG). Before

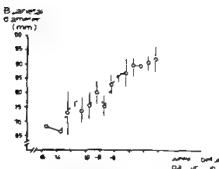


Fig. 2 The retrospective normal curve. The actual figures are given in Table II.

Table II Weekly average values of the biparietal diameter measured retrospectively from the time of spontaneous birth

Week	No of observations	Mean (mm)	S D
0.5	39	92.3	4.7
1.5	29	90.9	3.0
2.5	20	89.8	3.2
3.5	14	89.9	4.1
4.5	16	87.0	5.6
5.5	6	86.8	3.1
6.5	11	83.0	3.1
7.5	5	75.0	4.5
8.5	3	80.0	4.0
9.5	7	75.3	4.8
10.5	5	73.8	5.1
11.5	2	78.0	2.8
12.5	5	71.6	7.6
13.5	1	66.0	0.0
14.5	0	-	-
15.5	1	68.0	0.0

each measurement the apparatus was calibrated with a plexiglass cylinder which was constructed to give a distance equivalent to 2 cm in tissue. The velocity of the ultrasound beam was in the order of 1570 m/sec. The measurements were performed alternately by 3 observers. In a test series of duplicate measurements the standard deviation of the observer differences was 1 mm (?).

Statistical analysis The authors received help with recording and programming of the material for electronic data processing from the EDB section of the Medical Faculty, University of Bergen, and are especially grateful to programmer Alf M. Aksland.

RESULTS

The normal curves The prospective normal curve is presented in Fig. 1 and Table I. The number of observations after week 42 is small, but there is an indication of a decline in average values at this time.

The retrospective normal curve is shown in Fig. 2 and Table II. This curve is less smooth than the prospective curve. The cause for this is probably the smaller number of observations. The retrospective curve has a gradual increase up to the time of spontaneous delivery. When delivery (time 0) is set equal to 40 weeks of gestation, the retrospective curve is set equal to 40 weeks of gestation. The two curves are very similar.

Individual growth curves The individual growth curves, subgrouped according to the weight of the newborn child, are shown in Figs. 3-6. Cases

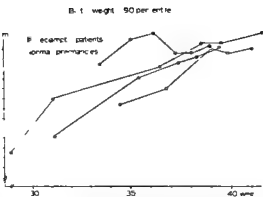


Fig 3 Serial measurements of the biparietal diameter in fetuses who were large for dates at birth (above 90th centile)

Maternal pre eclampsia can be identified by open circles in the figures. A comparison between the curves give the impression that there is a relationship between the biparietal diameter and the birth weight of the newborn child. However, this appears to be a statistical association rather than a clear-cut diagnostic index in the single case. This will be further discussed below.

Women with pre eclampsia had children in all birth weight groups, but their relative numbers increased with decreasing birth weights of the children. With a few exceptions the fetal skulls of pre-eclamptic mothers showed similar patterns to those of mothers without pre eclampsia.

The correlation coefficient between the biparietal diameter taken shortly before birth and the weight

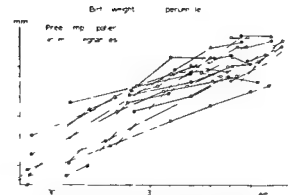


Fig 5 Serial measurements of the biparietal diameter in fetuses who were medium sized lower range at birth (between 10th-50th percentiles)

and length of the newborn child are shown in Table III.

DISCUSSION

The shape of the prospective curve of biparietal diameter growth of the present investigation is similar to that presented by Willocks et al. (8) with the exception of the slight decline following week 41. The differences in actual measurements between different normal curves is most likely to be due to calibration differences and not to reflect differences of fetal skulls. The dip following week 41 is a phenomenon which is also seen in normal curves of birth weight (3). The present material is made up of serial measurements of the same individual and

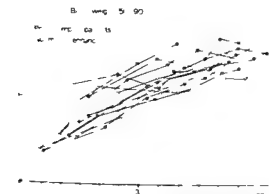


Fig 4 Serial measurements of the biparietal diameter in fetuses who were medium sized upper range at birth (between 50th-90th percentiles)

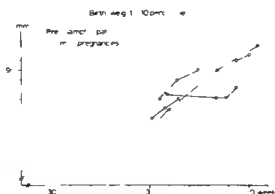


Fig 6 Serial measurements of the biparietal diameter in fetuses who were small for dates at birth (below 10th percentile)

Table III Coefficients of correlation (r) between the biparietal diameter measured during the last week before birth and birth weight and length

Condition	No of observations	Biparietal diameter versus birth weight (r)	Biparietal diameter versus birth length (r)
Normal pregnancies	58	0.58	0.72
Pre-eclamptic pregnancies	15	0.63	0.37

single measurements in other cases. The single measurements were more frequent towards the end of pregnancy at which time several women were referred to the obstetrical department for evaluation of hypermaturity. The shape of the curve is therefore thought not to reflect a true shortening of the biparietal diameter in individual fetuses. It is more likely to reflect a biological phenomenon which means either a preponderance of small fetal skulls among those going past term or an incorrect pregnancy dating on the part of the mothers. That the individual fetus does not show a shortening of the biparietal diameter is demonstrated in Figs 3-6 in which the trend of gradual growth up to the time of birth is evident. The few instances of shortening of the biparietal diameters with time are thought to represent either observer's error (2) or pathological conditions in cases of pre-eclampsia. The retrospective curve using spontaneous labour as the point of reference eliminates the possible error of correct dating but may include some cases of premature labour.

Individual growth curves grouped according to the weight-for-dates of the newborn child should indicate whether it is possible to predict the size of child at birth. Willocks et al (8) could predict the birth of a dysmature or a non-dysmature child with a fair degree of accuracy using a growth rate of 0.17 cm per week as the dividing line. We have not calculated the weekly growth indices but a glance at Figs 3-6 will show that such predictions would be of little value in these cases. A comparison of the large (Fig 3) and the small (Fig 6) babies shows that the former as a rule had a slower growth rate from about week 30 onwards than the latter. On the other hand the large babies had large biparietal diameters when first measured in contrast to the small ones. Thus the general trend was a con-

vergence of the curves towards a normal size with a smaller variation at term. The two groups, medium sized babies show considerable overlapping of the curves with a slight trend for the latter in the upper range to have larger biparietal diameters. The curves for fetuses of mothers with pre-eclampsia show the same growth trends as those mothers without disease with a few exceptions: arrested growth not thought to be due to measurement errors. In a previous communication Berg & Brodtkorb (1) found a more marked correlation between the biparietal diameter and prematurity than that of the present material. This first included many women with severe pre-eclampsia who had severely growth retarded children while the majority of the patients of the present material had mild pre-eclampsia.

The correlation coefficients (Table III) support the impression that there is no strong association between the biparietal diameter measurement and the weight-for-dates of the newborn child. The nutritional status of the fetus affects the body to a greater degree than it affects the head. On the other hand judged from the curves it seems that the time of maximum growth rate of the fetal head may be of prognostic significance. Fetuses destined to be large babies appear to have large biparietal diameters before week 30. Therefore a measurement of the biparietal diameter between week 20 and 30 in cases of known gestational age may prove to be the best prognostic index of the size of the newborn child.

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ALPHA FETOPROTEIN CONCENTRATION IN AMNIOTIC FLUID DURING THE LAST TRIMESTER IN NORMAL PREGNANCIES AND IN PREGNANCIES WITH SEVERE FETAL ABNORMALITIES

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Abstract The concentration of alpha fetoprotein (AFP) in amniotic fluid was studied during the last trimester of pregnancy. 179 samples of amniotic fluid were collected by transabdominal amniocentesis in 94 pregnant women. Only women with uncomplicated pregnancies giving birth to normal infants at term were included. The 90% reference interval was calculated and a distinct decrease in the amniotic fluid AFP concentration was found during the last trimester. An AFP concentration above the 90% reference interval was found in 8 out of 10 cases of anencephaly, normal AFP concentration was found in a case of congenital heart disease with severe oedema, and a low concentration was found in a case of Down's syndrome (Trisomy 21).

Increased alpha fetoprotein (AFP) concentrations in amniotic fluid in the last trimester have been reported in neural tube defects, in other severe malformations and in intrauterine fetal distress or prenatal death (1, 3, 5, 7, 11, 12, 14). The normal AFP concentration in amniotic fluid in the last trimester of pregnancy has not yet been clearly defined because of inadequate assay methods (1, 3) because the samples were not all obtained by transabdominal amniocentesis (1, 3, 11, 12) and finally because normality was not uniformly defined. The purpose of this study was primarily to estimate the 90% normal range for amniotic fluid AFP in the last trimester of pregnancy in normal pregnant women by means of a sensitive AFP assay. Secondly, the aim was to determine the AFP concentration in amniotic fluid from pregnant women with severe abnormalities of the fetus.

MATERIAL

Normal specimens

Transabdominal amniocentesis in 94 normal pregnant women in the last trimester. 179 amniotic fluid samples were

obtained (≥ 3 punctures in 7 cases, 2 punctures in 15 cases and 1 puncture in 72 cases). The punctures were performed between 9 and 12 in the morning. Specimens contaminated with meconium (greenish) were discarded. Only women with uncomplicated pregnancies and giving birth to normal infants of a birth weight ≥ 2500 g at term (38-42 gestational weeks) were included.

Pathological specimens

12 amniotic fluid samples were collected by transabdominal amniocentesis (5 cases) and by rupture of the membranes (7 cases) from pregnant women with abnormal fetuses (see Table 1).

METHODS

The amniotic fluid samples were all centrifuged for 10 minutes at 1500 g and the supernatants were kept frozen at -20°C until analyzed.

Measurement of AFP

Quantitative radioimmuno-electrophoresis (RIEP) as recently described (Nørgaard Pedersen, 1974) (9) was used for determination of AFP in amniotic fluid.

The RIEP gel plates were made by moulding a 1.5 mm thick gel between two gel plates. The gel consisted of two parts: the cathodic gel containing about 1 ng of ^{125}I AFP, the anodic gel containing 0.03 (v/v) volume fraction of AFP antibody (Antibody Rabbit immunoglobulins against human α_1 fetoprotein, Dakopatts, Copenhagen). Just before use of the plates a row of wells was cut in the cathodic gel near the border of the antibody gel. By means of a commercially available primary standard (α_1 fetoprotein standard 0.5 ml ^{125}I Behringwerke AG, Marburg/Lahn) a secondary AFP standard containing 640 $\mu\text{g/l}$ was prepared from a pool of amniotic fluids taken in the second half of pregnancy. This standard was used in dilutions from 640 to 20 $\mu\text{g/l}$. The wells were filled with exactly 15 μl of either samples or standards. After high voltage electrophoresis for 4 h or low voltage electrophoresis for 18 h the gel plates were dried and submitted to autoradiography for 74-48 hours using a common X-ray film (Kodak* RP/L, X-Omat

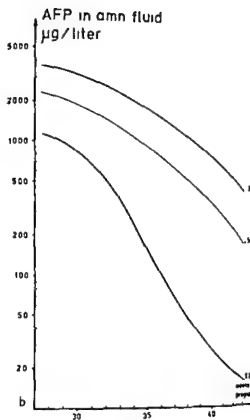
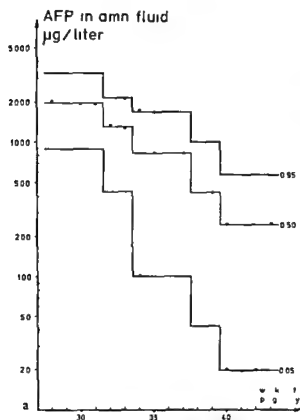


Fig 1 (a) The non parametric 90% reference curve for AFP in amniotic fluid from pregnant women in the last trimester of pregnancy (129 samples) (b) The correspond-

ing smoothed curve for amniotic fluid AFP in the third trimester of pregnancy

Table I Amniotic fluid AFP concentration in 12 pregnancies with severe fetal abnormalities

Transabdominal amniocentesis cases 1 3 7 9 and 11
Rupture of the membranes cases 2 4 5 6 8 10 and 12
H=high N=normal L=low

Case	Week	Newborn		Amniotic fluid AFP (µg/l)	
		Sex	Diagnosis		
1	40	♀	Anencephaly	890	H
2	35	♀	Anencephaly	1 280	N
3	39	♀	Anencephaly	147 000	H
4	36	♂	Anencephaly	79 150	H
5	29	♂	Acrania	53 500	H
6	34	♀	Acrania	8 900	H
7	33	♀	Anencephaly	20 700	H
8	35	♀	Anencephaly	700	N
9	40	♂	Anencephaly	15 200	H
10	39	♀	Anencephaly	2 270	H
11	36	♂	Anasarca m gr morbus cordis	1 300	N
12	40	♀	Down's syndrome	20	L

Intrauterine death of the fetus. The samples were collected before fetal death

Medical X ray film) in order to localize the rockets. In an LKB RCC 2105 spark chamber it was obtained a polaroid picture of the rockets within 10-20 minutes (17). The analytical coefficient of variation was calculated on the basis of 20 determinations on a pool of amniotic fluid samples with an AFP concentration of 380 µg/l. It was found to be 10.2%. The sensitivity of the method was 20 µg/l. Using this method one technician can perform 40 AFP measurements in duplicate.

Statistical method

A non parametric method was used for the computation of gestational age dependent reference intervals of AFP (Winkel & Jørgensen 1973). This method repeated binomial data of consecutive age groups. A computer program was used for the computation of the reference intervals. At each step gestational weeks with a mean difference between the location of the quantity and the location of the quantity are combined provided that the location of the quantity does not differ significantly with respect to the specified levels. The specified significance level of the study was 0.10 corresponding to a *P* value of 0.05. Reference intervals were computed for the resulting gestational groups.

RESULTS

Amniotic fluid AFP levels during the last trimester of pregnancy

The 90% tolerance interval for amniotic AFP during the last trimester of pregnancy is shown in Fig. 1 A. Fig. 1 B represents the corresponding smoothed curve. This curve was made by drawing the best fitting smooth curve to Fig. 1 A. A gradual decline in AFP concentration was found from the 27th to 40th week of gestation.

Amniotic fluid AFP levels from pregnant women with abnormal fetuses

In all cases of anencephaly except cases 2 and 8 significantly elevated AFP concentrations were found, whereas a normal concentration was observed in a case of congenital heart disease with hydrops oedema and a low concentration in a case of Down's syndrome (Trisomy 21) (Fig. 2).

DISCUSSION

In the computed non parametric curves in Fig. 1 and the smooth curves in Fig. 1 B it is seen that the normal range for AFP in amniotic fluid is rather wide and that a steady decrease is seen from the 27th to the 40th gestational week. The AFP concentration in amniotic fluid in this period is considerably lower than during the first half of pregnancy (9). A comparison between the present results and the results in other studies is difficult mainly due to differences in sampling procedure (puncture or rupture of the membranes immediately prior to delivery, vaginal amniocentesis, puncture of the membranes during cesarean section or transabdominal amniocentesis), differences in assay technique and in definition of normality and finally because of the use of different AFP standards (1, 3, 5, 7, 11, 12, 15).

In the present study all 129 specimens were obtained by transabdominal amniocentesis in 94 normal pregnant women all giving birth to normal infants. The general pattern for the AFP concentration in amniotic fluid in the last trimester of pregnancy as seen in Fig. 1 is similar to the pattern described by others (1, 3, 7, 11, 13). However the concentrations in relation to gestational age is more than twice as high as reported by Seppala & Ruuslahti (12, 14) and considerably lower than found by Brock & Sutcliffe (3). The reason for these dis-

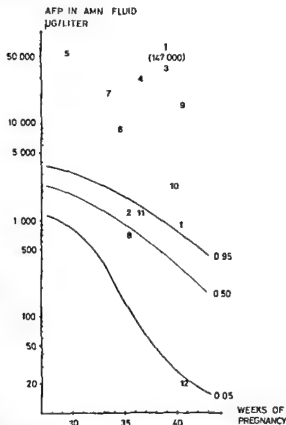


Fig. 2 AFP concentration in amniotic fluid from pregnant women with abnormal fetuses. The reference curves correspond to Fig. 1 A.

crepancies may be explained mainly by differences in standardization (14) and measurement. Seppala & Ruuslahti have found the Behringwerke standard about twice as high as their own standard (12, 14). AFP quantitation methods with a sensitivity of 20 µg/l or lower must be used for determining the normal AFP concentration in amniotic fluid in the second half of pregnancy. However, AFP assays with a sensitivity of about 200 µg/l (8) may be used to show whether the AFP concentration is above or within the normal range. This is important because these rather insensitive measurements usually can be finished within a few hours. From our measurements of AFP in amniotic fluid no close correlation has been found between the AFP concentration and gestational age as found by Seppala & Ruuslahti (14). However, AFP concentrations below 100 µg/l indicate that the pregnancy has advanced to at least the 33rd gestational week (see Fig. 1 A). The AFP concentrations found in amniotic fluid from pregnant women with abnormalities of the fetus are in agree-

ment with the findings in similar studies (1 3 5 7 11 12). The normal AFP concentration in amniotic fluid in 2 out of 10 cases of anencephaly do not exclude an elevated AFP concentration in the early pregnancy period in these two cases (cases 2 and 8). As shown in several studies it is especially during the first half of pregnancy that increased AFP concentrations in amniotic fluid is of practical importance (1 3 5 6 7). During the second half of pregnancy AFP measurements have especially been used to monitor severe Rhesus isoimmunisation and in the diagnosis of intrauterine fetal distress or death (11 12 13 15). Also in other types of fetal malformation than neural tube defects elevated AFP values have been found (11). It should be emphasized that amniotic fluid samples from Rhesus isoimmunisation patients could not be used for calculation of the 90% reference interval since increased AFP concentrations have been found in these patients (2 13 15). The diagnostic significance of AFP measurements in amniotic fluid in the second half of pregnancy has not yet been clearly defined. Similar measurements in maternal serum may be simpler and perhaps more acceptable in clinical practice.

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THE VALUE OF AMNIOTIC FLUID DIAMINE OXIDASE ESTIMATIONS IN THE MANAGEMENT OF SEVERE RHESUS ISO IMMUNIZATION

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act Serial estimations of the enzyme diamine ox (D A O) in liquor amni were made in 87 rhesus ised women. Normal levels were found in severe se with a favourable fetal outcome but abnormal s were found in 50% of the pregnancies in which e disease was associated with fetal death. In addi when liquor bilirubin levels predicted moderate se disease D A O levels were abnormal in 75% of the ancies in which the fetus succumbed. Abnormally D A O levels in amniotic fluid were frequently as ted with the subsequent development of dissemi l intra vascular coagulation in the infant. Serial estims of D A O in amniotic fluid could be a helpful ict to serial liquor bilirubin estimations in the man tent of severe rhesus iso-immunisation.

ough pennatal deaths due to rhesus iso- unisation continue to decrease in this country e remains the need to improve the prognostic lity of pre natal investigation in detecting not r the severely affected fetus but those which r survive with skilled obstetric and paediatric

stimation of the bilirubin concentration in liquor u is the recognised method of predicting the ntity of the condition in the fetus. However interpretation of the results may mis resent the severity of the effects of the disease he fetus leading to inappropriate management of

individual cases (6). Errors may also be due to technical difficulties (2) or inaccurate estimation of gestational age. Additional guidelines would help to eliminate these errors.

In an earlier report (9) we concluded that serial estimation of the enzyme in amniotic fluid might provide an indicator to the ultimate fetal outcome.

In this larger study the results of 331 D A O estimations in amniotic fluid from 95 women 82 of whom had rhesus iso-immunisation are presented.

PATIENTS AND METHODS

In patients with suspected rhesus iso-immunisation amniocentesis was performed on one or more occasions between 22 and 37 weeks gestation after ultrasound placental localisation. Five millilitres of maternal blood was taken at the same time. In addition fetal ascitic fluid was obtained at the time of intra-uterine transfusion on 35 occasions. D A O was measured using the method of Okuyama & Kobayashi (5) with modifications described by Southren (7). In order to provide a rapid method of estimation further modifications were made.

To increase the sensitivity the Carbon 14 was doubled and neat liquor samples were used (Normally a 1 in 10 dilution is employed). The incubation time was also reduced to 15 minutes and separation of aqueous layers was effected by standing for 5 minutes at room temperature before pipetting off the upper solvent layer for counting.

The D A O concentration in the serum and amniotic

Table 1 The mean values of amniotic fluid diamine oxidase in pregnancy after 25 weeks gestation complicated by rhesus iso-immunisation and in unaffected pregnancy

Severity of rhesus immunisation	No. of patients	No. of estimations	Geometric mean (mU/l)	Actual range
Affected	13	36	4.763	1.076-13.990
Unaffected	17	47	5.034	486-18.681
Total	17	67	6.671	2.009-17.270

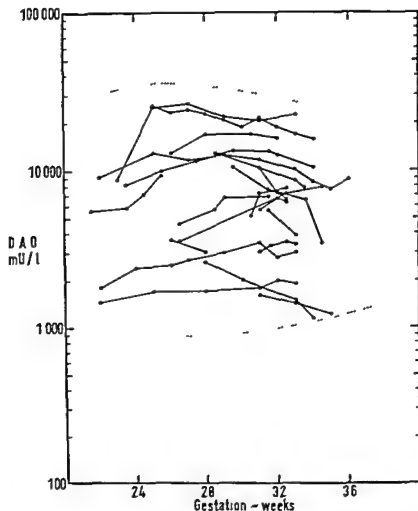


Fig 1 D.A.O. levels in amniotic fluid in 21 pregnancies of rhesus iso-immunisation in which the baby survived (8 estimations). The dotted line indicates the normal range of limits

fluid was expressed in mU/litres (8). The bilirubin content ($\Delta OD 450$) of the amniotic fluid was estimated by the method described previously (2).

The 95 cases were classified by severity of rhesus iso-immunisation into the following four groups

(a) 13 unaffected infants—Coombs test result negative (36 estimations)

(b) 17 infants with mild disease—Coombs test result

positive but exchange transfusion not needed (17 estimations)

(c) 17 infants with moderate disease—Coombs test result positive and exchange transfusion required, criteria for severe disease not satisfied (67 estimations)

and (d) 48 infants with severe disease (181 estimations) of whom were delivered alive with a cord haemoglobin less than 9 g/100 ml or a cord bilirubin of over 7 mg/l and 16 of whom were stillborn.

Of the 32 infants alive at delivery in this last group eleven died during the neo-natal period.

Table II Geometric mean of liquor diamine oxidase mU/l with 95 % limits ($t 0.05 \times S D$)

Unaffected (13 cases), mild (17 cases) and moderate (17 cases) (Includes only one point per patient per gestational group)

Gestation (weeks)	n	Geometric mean (mU/l)	95% limits
22-25	9	6 243	1 136-34 301
26-29	17	5 702	914-35 563
30-33	39	5 211	1 010-26 871
34-37	36	5 123	1 235-21 251

RESULTS

Amniotic fluid

The geometric mean levels of D.A.O. in amniotic fluid after 25 weeks gestation taken from unaffected pregnancies and those complicated by mild and moderate disease is shown in Table I.

In 2 cases of mild disease D.A.O. levels in liquor amni were consistently under 1000 mU/l.

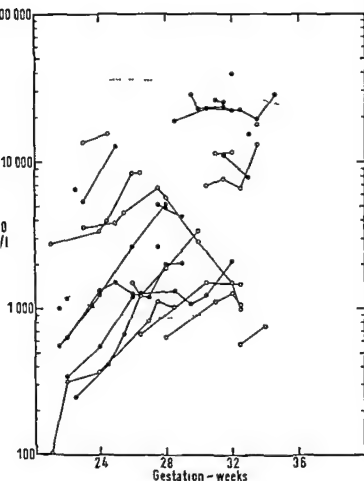


Fig 2 D A O levels in amniotic fluid in 27 pregnancies (11 N N D and 16 S B) in which the fetus succumbed (88 estimations)

in earlier studies we know that the D A O levels in amniotic fluid are reasonably constant in the last trimester (9)

Since statistically no significant differences were found between levels in unaffected pregnancies and those from pregnancies complicated by mild and moderate rhesus disease the 150 estimations were grouped together

The upper and lower 95% limits ($\pm 0.05 \times S D$) for this combined group at various gestational periods are given in Table II. This has been used as the normal range

The 48 severely affected pregnancies were considered according to fetal outcome. In 21 patients (estimations) in whom the fetal outcome was favourable there were no D A O levels in the amniotic fluid outside our normal range (Fig. 1)

The remaining 27 pregnancies (88 estimations) ended in either intra-uterine death (I U D)—16 pregnancies or neonatal death (N N D)—11 preg-

nancies. In 21 of these pregnancies in which serial D A O estimations were available 50% had one or more D A O levels outside the normal range (Fig. 2). In the remaining 7 pregnancies 3 or 43% of single estimates of D A O were outside the normal range

Using a University College Hospital modification of the Liley type prediction chart based on liquor $\Delta O D 450$ ($\Delta O D 450$ at 24 weeks < 0.3 at 28 weeks

Table III Summary of D A O levels and fetal outcome in pregnancies complicated by severe and moderate-severe rhesus iso-immunisation

Liley $\Delta O D 450$ prediction zone	Total no of cases	No of SB/NND	No of SB/NND with abnormal D A O levels
Severe	25	19	7 (37%)
Moderate-severe	2	8	6 (75%)

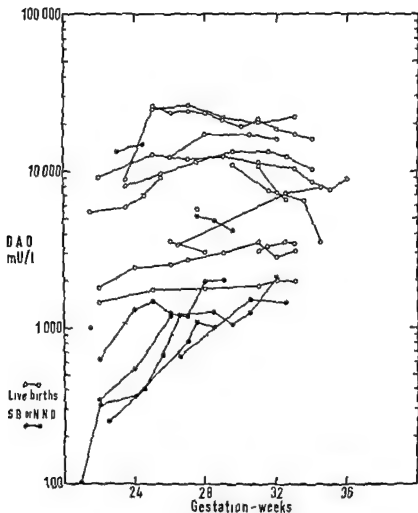


Fig 3 D A O levels in amniotic fluid in 77 pregnancies prior to delivery in moderate-severe range

< 0.22 at 32 weeks < 0.17 Fairweather et al (3)) 25 pregnancies were predicted as having severe disease. Seven (37%) of the 19 pregnancies in this group that ended in perinatal death had abnormal D A O levels in the amniotic fluid. In some of these cases abnormally high levels were found. However, of those predicted as moderate to severe there were 8 cases in which perinatal death occurred. In 6 of these (75%) the D A O levels in amniotic fluid were abnormally low (Table III and Fig 3).

One severely affected baby had bilirubin values predicting mild disease or an unaffected pregnancy and a favourable outcome associated with normal D A O levels.

Of the severe cases studied 15 had one or more intra uterine transfusions. No significant difference ($p < 0.07$) was found between the levels of D A O estimated before and after one or more transfusions.

Abnormally low liquor D A O levels (below 95%

lower limit) were associated with 5 of 6 babies which disseminated intravascular clotting (DIC) was diagnosed prior to NND. The levels in the sixth case were also low falling just within the lower 95% limit.

In 5 cases of NND where DIC was diagnosed despite routine neonatal screening D A O levels were within normal limits.

There were two surviving babies in which DIC was diagnosed. Liquor D A O levels in one were bordering on the lower 95% limit while in the other was mild and was associated with normal D A O liquor levels.

Amniotic fluid/serum D A O ratio

The ratio was investigated in the different cases of rhesus disease after 28 weeks when the ratio was expected to be constant (9). The mean ratio in each group are shown in Table II.

When the mean ratio of the unaffected group

Table IV Amniotic fluid/serum
lactate dehydrogenase ratio after 78 weeks gestation
Arithmetic mean \pm 1 S D

	No of cases	No of estimations	Arithmetic mean	S D
Unaffected	9	18	2.53	\pm 1.46
Mild	15	37	3.21	\pm 1.77
Moderate	16	50	4.80	\pm 4.28
Severe	35	98	5.07	\pm 3.09

compared with the mean ratios of the moderate and severe groups a statistically significant difference was present ($p < 0.05$ and $p < 0.001$ respectively). Similar findings were present when the mean ratio of the mild group was compared with the mean ratios of the moderate and severe groups. However no significant differences were found between unaffected and mild groups or between moderate and severe groups.

Ascitic fluid

D A O was estimated in 35 samples of fetal ascitic fluid obtained at intra uterine transfusion. The mean level was 33 mU/litre (range 0-123 mU/L). A comparison of D A O levels in amniotic fluid and fetal ascitic fluid taken from the same patient showed a statistical difference ($t = 4.8997$ $p < 0.001$ = 35).

DISCUSSION

Conventional tests of feto-placental function offer little guidance to the clinician in the management of severe rhesus iso-immunisation. Endocrine tests including the determination of maternal urinary oestrol, pregnanediol and chorionic gonadotrophin are of scant value (1) although the estimation of placental lactogen (10) may provide an indication of fetal outcome. There is little difficulty in detecting the very severely affected fetus using serial liquor analysis for bilirubin but the perinatal mortality is high despite expert obstetric and neonatal management. In this group abnormal D A O levels in the liquor were only found with an unfavourable outcome. However a normal level would not preclude a subsequent perinatal death. There was a surprisingly high perinatal mortality rate (36%) in the pregnancies in which moder-

ate-severe disease was predicted. Salvage of these babies is likely to be the most rewarding and serial estimations of D A O in the liquor would be of considerable value. Abnormally low levels were found in 75% of the pregnancies with perinatal deaths.

We were unable to confirm our earlier finding (9) that amniotic fluid/serum D A O ratio correlated with the Δ OD 450. Although the ratio increased with aggravation of the disease the scatter in the values of the ratio did not always reflect the fetal condition in individual cases.

It is of interest that liquor D A O levels did not appear to be affected by intra uterine transfusion suggesting that the procedure has an insignificant effect on the underlying disease process.

We are unable to offer an explanation for the association between low D A O levels in the liquor and the subsequent onset of disseminated intra vascular clotting (D I C) in the baby. Not only did 83% of the N N D with D I C have levels below the 95% lower limit but the D A O levels in liquor in 2 out of the remaining 3 cases with D I C had levels bordering on the lower 95% limit.

Is the detection of D A O in amniotic fluid of any value to the clinician?

Although the estimation of the liquor D A O does not necessarily detect the cases with severe disease with an unfavourable outcome normal D A O levels when severe disease is predicted would encourage the clinician to undertake intra uterine transfusion in circumstances in which active management may be considered hopeless. We like others (4) have found difficulty in diagnosing the degree of hydrops and anaemia in the fetus in utero an assessment which influences the timing of the initial intra uterine transfusion.

In the moderate-severe group the finding of abnormally low liquor D A O levels would alert the clinician to the probably poor fetal outcome. Similarly monitoring the coagulation factors in the newborn prophylactically in these cases may significantly reduce the perinatal mortality.

We have also found that the rapid method for estimation of D A O can be used to distinguish liquor amni from ascitic fluid. Blood staining of the fluid can make their differentiation otherwise difficult.

In conclusion we suggest that by using the simplified method for the estimation of D A O the measurement of serial levels of the enzyme in the

liquor routinely obtained for bilirubin analysis could make a considerable contribution to the planning of the pre natal and neonatal management of severe rhesus iso immunisation

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CASE REPORTS

EXTRAMEMBRANOUS PREGNANCY WITH AMNIORRHOEA

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Abstract A case of extramembranous pregnancy is reported. From about the 10th week of pregnancy there was intermittent loss of liquor amni which from about 24th week became continuous. The infant was born in the 31st week but died 15 days later because of respiratory distress syndrome. The placenta was circumvallate with short thick membranes unable to cover more than a small part of the infant. The course of pregnancy and the prognosis of the infant are discussed on the basis of previous publications. Because of the poor prognosis for the infant it is suggested that interruption of the pregnancy should be considered in early cases of amniorrhea.

mature rupture of the membranes with continuation of the pregnancy and retraction of the membranes after which the fetus develops partly inside the membranous sac has been known since the beginning of this century when Meyer Ruegg collected publications of 14 separate cases. In recent years several single cases have been reported (2, 4, 6, 10, 11, 12, 13). These cases are rare but of interest because of the problems arising

which continued until delivery. The fluid was examined for bacterial growth and α hemolytic streptococcus sensitive to penicillin were found. Penicillin (Vepicom bin®) was given without interruption until delivery. Once a week the fluid was examined bacteriologically and Gram negative rods—like Klebsiella—were found. They showed only slight sensitivity to a few antibiotics and specific antibiotic treatment was therefore omitted.

The genital region was kept as sterile as possible and no clinical signs of infection occurred.

The growth of the uterus continued normally and the values of urinary oestriol were rising.

By means of a Tassaway® rubber-cup the amount of vaginal fluid was estimated to be a maximum of 700 ml a day. The content of protein in the fluid was 3.9% which indicated that the fluid was a combination of liquor amni (protein content 0.2%) and plasma (protein content 7%). The serum protein content of the patient was 7.0%.

At 31 weeks gestation (on January 5, 1977) spontaneous labour supervened with a breech presentation. A live female child weighing 7050 g was delivered. Because of its low weight the child was immediately transferred to the pediatric department.

The placenta weighed 680 g. The membranes were short and thick with slight thickening of the free margin and fibrin at the base. The cord contained 3 vessels and there were no signs of infarction. The placenta was circumvallate (Fig. 1).

CASE REPORT

The patient was a 27-year-old gravida 2 who 2 years previously had had a normal pregnancy. She had a regular menstrual cycle and her last period began on June 3, 1975. After 10 weeks of amenorrhoea she was admitted to hospital because of brown vaginal discharge for 3 weeks and a loss of clear fluid vaginally for 7 days. The uterus was enlarged as expected and the cervix was normal. During an 11-day stay in hospital no loss of fluid was observed and the patient was discharged. During the following 7 months the patient several times observed a loss of clear fluid vaginally but examinations showed that the size of the uterus was compatible with the period of amenorrhoea and the cervix was closed. At 25 weeks gestation the patient was readmitted to hospital with a continuous loss of clear fluid vaginally.



Fig. 1 The circumvallate placenta showing the short membranes with the thickened edge.

Table I Data concerning rupture of the membranes duration of pregnancy and birth weight published in the recent years

Author and year of publication	Duration of pregnancy at membrane rupture (weeks)	Duration of amniorrhoea (weeks)	Duration of pregnancy (weeks) at delivery	Birth weight (gram)
Erskine 1945 (4)	18	17	35	2 000
Siddall 1946 (13)	18	13	31	1 950
Nold 1957 (10)	21	14	35	2 080
Rosset 1957 (12)	23	9	32	1 450
Carpenter 1965 (2)	20	15	35	1 950
Ringe 1967 (11)	23	7	30	1 400
Kohler & al 1970 (6)	16	11	27	1 100
Present case	11	20	31	2 050

The puerperium was uneventful. The treatment with penicillin was stopped at delivery.

Shortly after birth the child showed signs of the respiratory distress syndrome. It was treated with antibiotics and because of rise in the bilirubin values exchange transfusion was performed. Death occurred at the age of 15 days. Growth of *Klebsiella pneumoniae* was found at the time of death in the trachea and in the blood. Autopsy was not performed but no external malformation was seen.

DISCUSSION AND CONCLUSION

The intermittent loss of liquor amnii at the beginning of the pregnancy may be explained by a regeneration of the membranes which has been shown experimentally to occur in the guinea pig (14).

At the first admission to hospital there was doubt whether the fluid was liquor amnii or not. Examination with Nile blue sulphate is not helpful until after the 30th week of pregnancy (7) but when the fluid occurred in an amount large enough to collect estimation of the protein content suggested that it was a mixture of liquor amnii and plasma.

Most of the published cases of this kind are single cases. Table I shows some recent publications. It is seen that loss of liquor amnii in most cases occurred in the middle of the second trimester and premature delivery occurred in the middle of the third trimester. All the infants were alive at birth but died within a few hours except in the case described by Carpenter (2). This child survived and showed at the age of 17 months only slight retardation. The infant in our case survived for 15 days.

Two of the infants mentioned in Table I were malformed and in one child congenital luxation of the hip was found. Amnionitis occurred only in the

case described by Ringe (11). However premature rupture of the membranes does increase the risk of amnionitis and sepsis which increases with length of the amniorrhoea (1, 3, 5).

Both a reduced (1) and an unaltered (8) perinatal mortality have been described after using antibiotics in cases of premature rupture of membranes. To avoid infection it may be important to keep the genital region sterile.

As even symptomless cases of amnionitis may cause sepsis in the child in the neonatal period the infection with *Klebsiella pneumoniae* which was found before the death of the infant may have come from the *Klebsiella* like infection of the mother. The long interval (2 weeks) during which the child was treated with antibiotics however speaks against this. The placenta was circumferentially detached which is a common finding in these cases.

The poor prognosis for the child, the risk of infection and the emotional strain of a protracted stay in hospital are factors which may tempt the obstetrician to induce labour. Improved prophylactic treatment for infections and improved resuscitation of the premature baby may however improve the prognosis for the child but interrupting the pregnancy may be considered in early cases of amniorrhoea.

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TRISOMY D IN A CYCLOPS WITH CARDIOVASCULAR DEFECTS

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Abstract A case report of cyclops with trisomy D in a male infant associated with cardiovascular defects is presented. A survey of the literature pertaining to other reported cases of cyclopia is presented to establish the probability of the existence of at least two distinct ologic groups of cyclopia—those associated with trisomy D and those associated with other chromosomal aberrations, single gene mutations, or non-genetic factors.

The term arrhinencephaly described a wide group of facio-cerebral malformations, cyclopia being

considered the most severe anomaly of this type (12). This has recently been reclassified as a specific type of alobar holoprosencephaly (5).

Cyclopic malformation is characterized by a single central orbital cavity and a nose like tubular appendix situated above the orbit (19). To the best of our knowledge only 16 cases of cyclopia have been reported in which chromosomal studies had been performed (Table I).

In only four of these cases a normal karyotype was reported while among the others four distinct

Table I. Karyotypes and extracephalic defects in cyclops

Author	Karyotypes	Extracephalic and extrafacial malformations
Ikakı & Waxman (1)	47XX D+	Abnormal anal opening position (autopsy not done)
Ikakı et al. (2)	47XY D+	Polycystic kidneys, persistent omphalomesenteric duct, splenomegaly, persistent thyroid pyramidal lobe, cryptorchidism with hypoplastic testes, abnormally thick scalp and polydactyly
Johnson et al. (3)	47XX D+	Diaphragmatic hernia, dextrocardia, hepatosplenomegaly and hyperflexion of fingers
Johnson (4)	46XY/45XY G-	Renal anomalies, bilateral hydronephrosis, trigger thumb and short neck
Johnson & Lewis (6)	46XX 18p-	Hypoplastic adrenal and thyroid
Johnson et al. (7)	47XX D+	Polydactyly
Johnson & Cohen (8)	Normal	No information
Johnson et al. (9)	47XX D+	None
Johnson et al. (10)	Normal	No information
Johnson (11)	Normal	No information
Johnson et al. (13)	Normal	No information
Johnson et al. (14)	46XX 18p-	None
Johnson et al. (15)	47XX D+	Polydactyly
Johnson (16)	46XY/47XY	No information
Johnson & Tinaztepe (17)	47XY D+	Patent ductus arteriosus, atrial septal defect, dextrocardia, coarctation of aorta, malrotation of intestines, Potter type III, polycystic kidney, ectopic splenic tissue and polydactyly
Johnson & Jones (18)	47XX D+	Ovarian agenesis and hyperflexion of fingers
Johnson case	47XX D+	High interventricular septal defect, hypoplastic pulmonary artery, transposition of great vessels and hyperflexion of fingers

anomalous karyotypes have been described (Table I). Trisomy D has been found in eight of these cases, two of which were associated with cardiovascular defects (17/3).

In this report we present an additional case of trisomy D in a cyclops with cardiovascular defects.

CASE REPORT

The infant was born to a 41 year-old white American woman after a gestation of 36 weeks. Three previous pregnancies resulted in a macerated fetus and two normal children (one male and one female). In the present pregnancy toxemia and hydramnios were noted during the last month of pregnancy prior to delivery. There was no history of familial congenital malformations, parental consanguinity or X-ray exposure. Drug ingestion was limited to the last month of pregnancy for the treatment of the toxemia. No other drug therapy was reported during pregnancy.

The propositus was born by spontaneous delivery after a short labor. Physical examination at birth revealed a female infant weighing 1800 g and measuring 46 cm in length with an Apgar Score of 5. The baby showed the



Fig 1 Close up of face showing the characteristic features of cyclops malformation.



Fig 2 Coronal section of brain showing a single ventricle with no signs of separation.

characteristic features of cyclops malformation: a central orbital cavity with a rudimentary palpebral fissure on either side. Above the orbit a tubular appendix with a blind opening resembling a nose was found (Fig. 1). Hyperflexion of fingers was observed as well.

The infant died 55 minutes after delivery.

PATHOLOGY

Upon post mortem examination defects were covered in only two organ systems: the central nervous system and the cardiovascular system. Examination of the CNS revealed a brain one-third the normal weight for its age (83 g cf 255 g normally). Brain dimensions were also small: 6 cm long, 3 cm wide and 4 cm high. The two cerebral hemispheres were fused and formed a single mass—except for a delineation in the posterior-occipital region (for a length of 2.5 cm). The gyri were flattened and the outer surface of the brain showed marked congestion.

Upon examination of the base of the brain, olfactory bulbs, optic nerves and optic chiasm were found to be absent. Coronal section of the cerebrum revealed a single ventricle with no signs of separation (Fig. 2). The inner surface of the ventricle was smooth and glistening. The corpus callosum and septum pellucidum were not detectable. The brain tissue surrounding the single ventricle was 0.8–1.2 cm wide and there was no clear separation between grey and white matter. The posterior-caudal part of the aqueduct, the cerebellum, fourth ventricle and the medulla oblongata were normal.



Fig 3 karyotype from cultured blood showing trisomy D (The Sheba Medical Center Cytogenetics Laboratory Tel Hashomer)

The cardiovascular system defects included high interventricular septal defect, hypoplastic pulmonary artery and transposition of the great vessels.

CYTOGENETIC STUDIES

Immediately upon death, a blood sample via transudic puncture was taken for chromosomal analysis. The resulting leucocyte cultures revealed a chromosome count of 47 in all 30 cells of the culture. The additional chromosome was of the

D-group. The sex chromosomes were XX. This is a variant of trisomy D, 47,XX,D+ (Fig 3).

DISCUSSION

Of the various karyotypes associated with cyclopia (Table I), trisomy D has been described with the greatest frequency, and this form of cyclopia is the only one which is probably of the type of facio-cerebral malformations described by Kundrat (12) as arrhinencephaly. This evidence strongly suggests the possibility of classifying this malformation

into two distinct causal groups. In one group the causative factor may be chromosomal aberrations other than trisomy D or in the case of normal karyotypes some unknown entity. In the other group trisomy D is the causative agent. In our case this theory is supported by the cardiovascular findings which are characteristic even in non cyclopic cases of trisomy D.

It has been accepted that extracephalic malformations are present in arhinencephaly with trisomy D. In all but one of the reported cases of cyclopia with trisomy D extracephalic malformations have been found. This report by Halbrecht et al (9) seems to question the validity of the above classification by describing a case without such findings.

The extracephalic malformations described in cyclopia associated with trisomy D show no uniform pattern; however polydactyly also a typical finding in non cyclopic trisomy D has been described in 4 out of 8 cases (Table I).

In conclusion it is our conviction that arhinencephaly with trisomy D and cyclopia with trisomy D are related entities with trisomy D being the common etiologic factor. The second group including cases of cyclops either associated with normal karyotypes (etiologic factors of which remain to be discovered) or with chromosomal aberrations not of the D group most probably belong to different types of facio cerebral malformations.

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OPEN RUPTURE OF THE SYMPHYSIS PUBIS DURING SPONTANEOUS DELIVERY

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Clinical and roentgenological picture of symphysiolysis is very well known. In contrast the syndrome of the rupture of the symphysis pubis during spontaneous delivery is less well known. We herewith report a case of rupture of the symphysis pubis during spontaneous delivery.

very scarce. The opinions about the frequency of this phenomenon are divided. Cibils (2) cites Boland (1934) who found a frequency of 1 rupture to 521 deliveries, and Eastman (1966) who found a frequency of 1 rupture to 20 000 deliveries.

The etiology of the rupture is not very well

CASE REPORT

A 27-year-old woman para 1 gravida 1 was admitted to the Obstetric Ward on the 27th of June 1973 at the beginning of her labour. Her pregnancy had been normal. 8 hours after admission she was delivered of a boy weighing 3850 g. A right medio-lateral episiotomy had been performed before delivery. After the delivery of the placenta the patient complained of a sharp pain in the region of the symphysis. At examination a rupture of the vagina was found. The rupture started above the urethra. The bladder and the urethra were drawn into the vagina. Through the rupture the pubis bone were seen at an interval of 3.5 cm between them. Between the ruptured perineum was seen (Fig. 1). The ruptured vagina was sutured with the replacement of the urethra and the bladder. The orthopaedic treatment of the ruptured symphysis was a fixation of the pubic bones aided by tight pelvic binder during 3 weeks. A Foley catheter was inserted into the bladder for 12 days. The patient received also antibiotic and analgetic therapy. Three weeks after complete bed rest she had still some difficulties and pains in the retropubic area. An examination of the pelvis showed the almost complete disappearance of the rupture. Five months after the rupture she had completely healed (Fig. 3).

DISCUSSION

Literature discussing separation or rupture of the symphysis pubis during spontaneous delivery is



Fig. 1



Fig 2

known yet. As is well known, progesterone and relaxin heighten the degree of elasticity of the ligaments of the pubis bone. According to Trillat et al (3), the limit of elasticity during delivery is of 25–35 mm; when this limit is exceeded, the ligaments rupture, especially the anterior one, and then the rupture of the symphysis occurs. According to the same author, this phenomenon is more frequent in multiparas.

The treatment is administered according to two policies: (a) conservative, (b) surgical.

According to Cibils (2), absolute bed rest and tight pelvic binding seem to ensure complete recovery. According to Trillat et al (3) and Charvet et al

(1), the treatment has to be conservative by mobilization and hammock suspension during a period of three weeks, when the space between two pubis bones, according to X-ray, does not exceed 40 mm.

In contrast, in the cases in which the obstetric pubic distraction is more than 40 mm, the treatment has to be surgical: osteosynthesis of the pubis, with the aid of metallic cerclage.

CONCLUSIONS

The syndrome of the ruptured symphysis pubis during spontaneous delivery is very rare. Today,



Fig 3

vere complications of delivery which were reported 30 years ago are no more seen. In our case complete bed rest for 3 weeks and fixation with tight pelvic binding resulted in complete recovery.

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ACTIVE PRE TERM MANAGEMENT OF SEVERE OSTEOGENESIS IMPERFECTA

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Abstract Some of the advantages of using an intra amniotic injection of urea combined with prostaglandins and thimster termination include an extremely high success rate a mean time of approximately 10 hours and recovery of a dead fetus. The successful application of this method for the active pre term management of patients in whom severe fetal abnormality such as that reported here suggests this technique of inducing abortion and labour deserves further consideration in those special circumstances where the obstetrician is anxious that the pregnancy should be interrupted despite the gestation length at which a live birth should not occur.

In recent years termination of pregnancy in the first and second trimester has been increasingly performed on patients in whom suspected or proven fetal abnormality has been demonstrated as a result of specific investigations including chromosome patterns of amniotic fluid cells, alpha-fetoprotein levels, amniocentesis, amniography and rubella antibody titres. Although the range of abnormalities which may be detected in early pregnancy is likely to increase, the defects may not become apparent until late pregnancy and then present the obstetrician with a decision as to whether to actively intervene or to leave the situation alone despite the emotional stress that the latter decision might engender. This report describes the active management of a patient in whom severe osteogenesis imperfecta was diagnosed at 32 weeks.

CASE REPORT

A 34-year-old woman aged 40 years booked for confinement at 10 weeks gestation having had a normal delivery 10 years previously. Amniocentesis was performed under ultrasound control for alpha-fetoprotein levels and

chromosome constitution because of her age. The alpha-fetoprotein levels of the serum and liquor amnii were normal but poor growth of amniotic fluid cells prevented any conclusive report on the genetic status of the fetus.

Serum ultrasound assessment of biparietal diameter measurements indicated a satisfactory fetal growth rate but the fetus was reported to be difficult to define. The antenatal care remained uncomplicated until 37 weeks when polyhydramnios rapidly developed. The biparietal diameter at this time was 8.45 cm and an apparent double echo was seen. A straight X-ray of the abdomen showed that the only fetal parts visible were vague soft tissue outlines and parts of the vault of the skull. The appearance was consistent with an extreme case of osteogenesis imperfecta.

At 34 weeks one litre of amniotic fluid was aspirated because the patient became extremely uncomfortable. Within 48 hours the situation had become exacerbated and difficulty with respiration was experienced. The patient was aware both of the diagnosis and the prognosis of the condition and a decision was made to terminate the pregnancy in view of the predictable outcome and the degree of polyhydramnios.

TECHNIQUE

Vaginal examination prior to the procedure revealed a long uneffaced cervix 3 cm dilated. Amniocentesis was performed using a supra pubic drainage cannula (Bard International No SP1966) passed into the amniotic cavity under local anaesthesia with the bladder previously emptied. One litre of amniotic fluid was aspirated and then a solution containing 80 g urea (Ureaphil Abbot Laboratories) made up with 80 ml sterile Hartmann's solution was injected and followed by PGF₂ 5 mg as described previously (1).

Uterine activity developed rapidly and the patient was sedated with intramuscular diamorphine 10 mg. A grossly abnormal stillborn female infant weighing 2404 g was delivered 1 hour 46 minutes later.

The external and radiological features of the fetus are shown in Figs 1 and 2 respectively. The findings on post



Fig 1 External appearance of fetus exhibiting severe osteogenesis imperfecta

Mortem examination included marked scalp oedema, shortened limbs with very prominent skin creases, bowing of the legs and a small thorax containing hypoplastic lungs. The bony skeleton showed pronounced generalised osteoporosis with shortening and deformity of the long bones, a softened cranial vault, healed fractures and normal epiphyseal cartilages.

Tubal ligation was performed on the third day and the patient made an uneventful recovery.

DISCUSSION

Osteogenesis imperfecta is an inherited autosomal dominant disease with a high rate of spontaneous mutation presenting in varying grades of severity with defects in collagen containing tissues, particularly involving skeletal bones (1-3). Less severely affected cases have thin bones which fracture after birth. Deafness and blue sclera may be other man-

ifestations. More severely affected cases like one described here survive only a short time, have multiple fractures and other abnormal features such as phocomelia. Associated polyhydramnios may be a feature (4).

A decision to induce labour before term in a patient with severe fetal abnormality can only be contemplated if the outcome of induction of labour can be guaranteed. The technique of using carbonyl intra amniotic urea and PGE_2 as opposed to other alternative methods e.g. extra amniotic prostaglandins or more standard approaches such as caesarean section and intravenous oxytocic stimulus was selected because of the very high success in terminating mid trimester pregnancies found with this method and because of the likelihood that severely affected fetus would not be born.

Fig 2 X ray of the fetus showing osteogenesis and deficient bone formation

alleviating additional distress. Although live fetuses have occurred following induction of mid-trimester abortion using intra amniotic hypertonic saline and other agents, none have been observed with the standard dose of urea and variable amounts of PGE_2 which have been used to successfully induce abortion without failures in over 130 cases. The dose of PGE_2 administered was empirically selected as twice that currently being used for mid-trimester termination because of the possible diluting effect of the excess liquor present. The experience obtained of using this method for induction of abortion in patients with other severe abnormalities incompatible with life, including anencephaly, suggests this may be the treatment of choice in such situations.

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ANNOUNCEMENTS

Symposium on Medical Genetics The Hungarian Society of Human Genetics will organize an International symposium with WHO support in Debrecen Hajdusoboszló Hungary 27-29 April 1976 Topics Cytogenetics population genetics prenatal diagnosis and genetic counselling chromosomal mapping clinical genetics on the molecular level hemoglobinopathies immunogenetics Further enquiries should be addressed to Human Genetic Laboratory H-4012 Debrecen P O Box 37 Hungary

XXIVth Annual Colloquium on Protides of the Biological Fluids is due to be held in Brugge 27 April-1 May 1976 Topics Pregnancy associated proteins carcino fetal and carcino placental proteins detection and quantitation of proteins by molecular amplification Further information through Secretariate Simon Stevin Instituut Jerusalem straat 34 B 8000 Brugge Belgium

International Congress of Sexology Montreal 28-31 October 1976 Program Sex therapy sex education sexual dimorphism sex difference in erotism sexological aspect of contraception problems of ethics in sex therapy and sex investigation Co-sponsors Dept of Sexology of the University of Quebec in Montreal and Society for the Scientific Study of Sex USA Further information Pro-

fessor Robert Gemme Department of Sexology University of Quebec in Montreal C P 8888 Montreal Quebec Canada H3C 3P8

C S Mott Center Colloquium in Andrology The C S Mott Center for Human Growth and Development is holding a colloquium on *Techniques of Human Andrology* May 6-7 1976 at Wayne State University School of Medicine Detroit Michigan Research papers on techniques are accepted before February 1 1976 Invited papers will deal with evaluation of testicular biopsy physiology biochemical and bacteriological tests of semen chemistry of spermatozoa histochemistry of male organs ultrastructural localization of enzymes enzyme profiles and karyotyping of men clinical examination of infertile men biochemical and hormonal evaluation of prostate post coital tests freezing of sperm artificial insemination and behavioral therapy of male sexual inadequacies Films Sexual Therapy of Male Sexual Inadequacies panel discussion (analysis of male sexual pause and erectile and ejaculatory disorders) For information write to Dr E S E Hafez Department of Gynecology-Obstetrics 550 E Canfield Detroit 48201 (Tel 313/577 1011) Registration before March 1976 is for limited number

LETTER TO THE EDITOR

the article 'Study of glucose-6 phosphate and lactate dehydrogenases DNA RNA and total protein in the rabbit placenta during its hypertrophic response to ovariectomy' (Acta Obstet Gynecol Scand 53 209 1974) the authors state that the increase in placental weight is apparently due to hypertrophy rather than to hyperplasia as indicated by unchanged DNA. The data however seem to support an alternative explanation.

A review of the data shows that the DNA/g of wet weight (and presumably cell number) was similar in both control and experimental placentas as was the total nitrogen. The DNA/total nitrogen ratio per gram placental weight is therefore unchanged. Since there is no evidence of cellular hyperplasia. Furthermore since the placental weight was increased in the experimental group the total DNA (and hence cell number) will be higher indicating cellular hyperplasia without hypertrophy accounts for the increase in placental size.

It should be mentioned parenthetically that the number of the total number of fetuses or liveborn per litter for each group is not included in the results. If there is a negative correlation between litter size and placental (and fetal) size it follows that any comparison of placental weight the mean number should be similar in the experimental and control groups. An evaluation of this point is possible. However if the number of placentas is taken as representing litter number then the mean number of fetuses per litter in the experimental group would be 4.6 (37-8) vs 6.7 (47-7) in the controls. A difference that could account for the larger placentas in the former group. It is fully realized that the above calculation is based on an

assumption and hence may be in error but it serves to underscore the importance of taking litter size into consideration.

That bilateral ovariectomy (probably prior to day 27) in pregnant rabbits leads to larger placentas (and fetuses) is now well established. Our data show that the increase in placental size is limited to the fetal placenta and that cellular hyperplasia without a demonstrable change in cell size occurs. We have further shown that this response is a function of the estrogen milieu (1-4).

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APPEAL

Information is required on a hypothesis concerning the sex of human zygotes. For this purpose will anyone with data on the sex of any monoamniotic twin pairs please communicate with Doctor W James Galton Laboratory University College London W C 1 England

This appeal has been received from Doctor James who has an interesting theory concerning the formation of the sex of the conceptus and needs material for his research in order to prove his hypothesis

FIGO NEWS

INTERNATIONAL FEDERATION OF GYNECOLOGY AND OBSTETRICS (FIGO)

Secretariat Maternité 1211 Geneva 4 Switzerland

ABSTRACTS

Minutes of the 22nd annual meeting of the Executive Board of the International Federation of Gynecology and Obstetrics held on Sunday March 2nd 1975 in the Aqueduct Hall of the West End Hotel Bombay India

Attendance

24 Officers and Executive Board members 19 were present or represented

Officers (with special status) Dr B N Purandare (India) President Dr A Ingelman Sundberg (Sweden) Vice President Dr R Caldeyro-Barcia (Uruguay) President elect represented by Dr C MacGregor (Mexico) Dr P Magnun (France) Treasurer represented by Dr de Watteville Dr H de Watteville (Switzerland) Secretary General Dr R Vokaer (Belgium) Deputy Secretary General Dr H A Kaminetzky (USA) Editor of the FIGO Journal—excused

Non rotating members Dr J Varangot (France) represented by Dr C Bureau Dr H Husslein (German Democratic Republic) Sir Stanley Clayton (Great Britain) Dr L Jhaveri (India) Dr S Sakamoto (Japan) Dr L Telazo-Ayala (Mexico) represented by Dr C MacGregor Dr K P Russell (USA) represented by H C Taylor Dr L S Persianinov (USSR) represented by Dr T V Chervakova

Rotating members Sir Lance Townsend (Australia) Dr A Salvatore (Brazil)—excused Dr C Gomez Rogers (Belgium)—absent MUDr A Kotasek (Czechoslovakia) Dr H Mossadegh (Iran) Dr G J Kloosterman (Netherlands)—excused Dr O Akintola (Nigeria) Dr S S Ram (Singapore) Dr A Dominguez Gallegos (Mexico)—absent

Special committees Dr C P Hodgkinson (USA) Chairman of the Committee on Annual Reports and Minutes of Terms in Human Reproduction—excused H Ulfelder (USA) Chairman of the Cancer Committee of FIGO—excused Dr R Contamin (France) Chairman of the Joint Committee for the Study of Gynecological Problems in Childhood and Adolescence represented by Dr L Rauramo (Finland) Dr S Tomkinson (Great Britain) Chairman of the Joint Study Group on the Training and Practice of Midwives and Maternity Nurses—present Dr A Alvarez Bravo (Mexico) Chairman of the Committee on Maternal Mortality Studies—excused Dr H C Taylor (USA) Chairman of the Committee on Medical Aspects of

Human Reproduction—present Dr G Resau (Italy) Chairman of the Committee on Perinatal Mortality—excused Dr P O Hubinont (Belgium) Chairman of the Committee on Qualifications—absent Dr F G Geldenhuys (South Africa) Chairman of the Committee on the Reorganization and Future Functions of FIGO—absent Dr O Kaser (Switzerland) Chairman of the Scientific Program Committee for the VIIth World Congress—excused

Invited Dr P Muller (France) was invited to participate in the session when the item relating to gynecological problems in childhood and adolescence was discussed

2 Agenda

The provisional agenda was adopted after addition of item a) Discussion of the draft amendments to the Bye Laws of FIGO concerning the Special Committees of the Federation and "FIGO's Relations with other international organizations" to point 4 of the agenda Secretariat of FIGO

3 Minutes of the 21st annual meeting of the Executive Board

The Minutes of the 21st session of the Executive Board held in London on July 6th 1974 were adopted with one minor change in wording related to Sir Lance Townsend's active participation in the work of the Perinatal Mortality Committee

4 Secretariat of FIGO

Report by the Secretary General Dr de Watteville (Switzerland) Secretary General of FIGO read his report on the activities of the Secretariat

In his report the Secretary General expressed appreciation to Dr Vokaer and his co-workers at the French speaking sub-secretariat of FIGO in Brussels for their most valuable cooperation thanks to which all relevant documents have been translated into French and dispatched to the 25 Sociétés wishing to receive FIGO's documents in this language For information Dr Vokaer's address is as follows Service de Gynécologie et d'Obstétrique Hôpital Universitaire Brugmann 4 place A van Gehuchten 1020 Brussels Belgium

The Secretary General furthermore added that the list of Heads of University Departments of Obstetrics and Gynecology and Directors of Research Institutes dealing with Human Reproduction is under revision and will be

ready on time for the VIIIth World Congress in Mexico City October 1976. This document and the list of constituent Societies of FIGO with their addresses and the names of their Presidents and Secretaries are available at FIGO's Secretariat in Geneva.

Links have been maintained with the World Health Organization as in the past and thanks were expressed to those colleagues who represented the International Federation at regional and Expert Committee meetings organized by WHO.

Draft amendments to the Bye Laws of FIGO The draft amendments concerning the Special Committees of the Federation and the FIGO's Relations with other international Organizations were submitted by the Secretary General. Both drafts were accepted without discussion and modification and will be submitted for ratification to the General Assembly in Mexico City.

Committee on the Reorganization and future Functions of FIGO No report was received from Dr Geldenhuys (South Africa) Chairman of the Committee who was prevented from attending the meeting.

The Secretary General read two letters received from Executive Board members and three from affiliated Societies in reply to Dr Geldenhuys' report which had been dispatched to FIGO's constituency after the last Board meeting in July 1974.

Further to a long discussion concerning the replacement of the Secretary General and after a formal vote it was decided that a small special Committee of Board members familiar with FIGO's activities and problems should after a thorough investigation set up a list of candidates for the post of Secretary General and prepare a detailed report on the problem of this succession to be handed over to the Nominating Committee as a guideline for its deliberations. The following Board members were designated for this Committee: Dr Ingelman Sundberg (Sweden) Vice President of FIGO as convener, Dr Geldenhuys (South Africa) Chairman of the Committee on the Reorganization and future Functions of FIGO, Stanley Clayton (Great Britain) and the representative France.

5 *International Association for Maternal and Neonatal Health*

A report including a new draft for the Constitution of the International Association which will be formed by national sections as well as individual members was read by the Secretary General.

The report was adopted and the possibilities for the Board members to help organizing the International Association were discussed. The independence of the International Association vis à vis FIGO was stressed.

The four members on the Scientific Council of the International Association to be designated by FIGO were nominated: they are Dr Purandare (India), Dr Alvarez Bravo (Mexico), Dr Sakamoto (Japan) and Dr Sureau (France).

6 *Nominating Committee*

According to the Bye Laws the Nominating Committee for election at the next World Congress in Mexico City of the new Officers, three rotating Board members and the

Chairman and members of the Scientific Committee for the IXth World Congress was appointed.

Further to the following ex-officio members, 2 Board members and three delegates of Societies represented on the Board were to be designated:

Ex-officio members: 1) Dr B. N. Purandare (India) President of FIGO, 2) Dr R. Calderón (Uruguay) President-elect, 3) Dr H. de Witz (Switzerland) Secretary General.

After a secret ballot the following Board members were selected: 4) Dr S. Sakamoto (Japan), 5) Dr K. F. I. (USA), 6) the representative of France.

The following Societies were chosen for their delegate: 7) Peru, 8) Philippines, 9) Poland.

The choice was made taking into consideration representation on a Nominating Committee and geographic distribution.

7 *Finances of FIGO*

The report of the Treasurer, Dr Magnin (France), presented by his proxy Dr de Watteville (Switzerland).

As of December 31, 1974, the financial situation showed a credit balance of SF 237 014.70 which means an increase of SF 74 000 since December 31, 1973. However, the increase is mainly due to the contribution of SF received from the Organizing Committee of the IXth World Congress in Moscow. In 1974, FIGO spent 64 298.05 for its activities (Secretariat and Special Committees).

The budget for 1976 as suggested by the Treasurer amounting to SF 78 000 was adopted without discussion.

In his report the Treasurer expressed his concern about the devaluation of the US dollar since the contributions of FIGO's constituency are paid in this currency and expenses mostly in Swiss francs. Therefore he suggested that possibly the annual contribution should be increased to US \$150 per active member at the next General Assembly in order to face the continuous devaluation of the dollar. The Secretary General pointed out that the devaluation of the Swiss franc is most likely to occur in the near future. After discussion it was decided to propose to the General Assembly an increase in the membership fee for the time being.

The Treasurer's report shows that on the whole contributions of the affiliated Societies are paid within admissible time limits. However, there are Societies—grouping a small number of active members—which have not fulfilled their financial commitments towards FIGO for three or more years despite repeated reminders sent out by the Secretary General. (Three from Latin America, two from Africa, from Asia and one from Europe). According to a decision taken by the last General Assembly, these Societies will be formally excluded from FIGO's constituency now. However, the Secretary General suggested that those Societies which failed to pay their dues by December 31, 1975 should not enjoy the right extended to the members of affiliated Societies to participate in FIGO world congresses, to pay registration fees, right to present a voluntary communication, participation of a delegation of the Society in the General Assembly. This suggestion was accepted.

11th World Congress of Obstetrics and Gynecology Mexico City October 17-23 1976

Organization of the Congress Dr MacGregor (Mexico) Secretary General of the Congress substituting for Dr Estelazo-Ayala President of the Congress presented the progress report of the Organizing Committee while the rest of the report was being distributed to the Board members present

The members of the Board expressed their deep appreciation for the excellent work accomplished by Mexican colleagues and Dr Husslein (Australia) representative of the German Federal Republic thanked him especially for the inclusion of German as an official congress language during the main sessions The Secretary General expressed the satisfaction of the Board at Spanish English simultaneous translation will be available for all seminars and voluntary contributions

The registration fees as proposed by the Organizing Committee were accepted they are up to April 15 1976
 • \$130 00 per active participant US \$80 00 per accompanying member after April 15 1976 US \$150 00
 • active participant US \$100 00 per accompanying member

It was furthermore specified that Congress participants belonging to an affiliated Society will pay an increased registration fee as follows up to April 15 1976 US \$200 00 per Congress participant US \$100 00 per accompanying member after April 15 1976 US \$170 00
 • Congress participant US \$170 00 per accompanying member

The schedule for the meetings of the special committees October 14 & 15 1976 as prepared by the Secretary General was discussed In spite of the inconvenience for committee members to reach Mexico City three days before the opening of the Congress it was felt that there was no better possibility The Secretary General was asked to inform the chairmen of the special committees as far as possible and request that they confer this information to their committee members

The Secretary General reported on his correspondence with the liaison officers of the International Society for the Study of Trophoblastic Disease and the International Society for the Study of Vulvar Disease Drs Goldstein di Paola and with Dr Ulfelder Chairman of the Executive Committee of FIGO relating to joint meetings with ISSTD and ISSVD will hold a meeting in Mexico City starting October 24 and therefore it would be possible to hold a joint meeting with each of these groups on Saturday October 23 after the Closing Ceremony of the FIGO World Congress Papers could be read on invitation by members of FIGO as well as members of ISSTD and ISSVD and Congress participants could attend the meetings by prolonging their stay in Mexico City of a few hours only

Dr MacGregor pointed out that it was already planned to have a correlated seminar on trophoblastic disease and this could possibly be transformed into a joint meeting Another possibility would be to hold the meetings on Wednesday afternoon as no other scientific activities are scheduled at that time While giving its agreement on the principle of the joint meetings the Board asked the Secretary General of the Congress to make the

necessary arrangements as he deems it best after having discussed the matter with Dr Goldstein for ISSTD and Dr di Paola for ISSVD as well as with Dr Ulfelder Chairman of the Cancer Committee of FIGO

The suggestion of the Secretary General to offer the Presidents of ISSTD and ISSVD an opportunity to deliver a speech during the last main session of the Congress on Saturday morning (October 23) was not retained because the time schedule appeared already tight and the fear was expressed that this would create a precedent and that other small groups could expect the same privilege in future

Scientific Program of the Congress The scientific program as proposed by Dr Kaser (Switzerland) Chairman of the Committee was accepted with only two minor changes

It was decided that all voluntary contributions should be screened by the national Society to which the author is affiliated March 31 1976 was accepted as deadline for handing over the abstracts of the papers announced to the local Organizing Committee

A special Seminar dealing with teaching of social obstetrics will probably be organized by the WHO Unit of Maternal and Child Health As the WHO budget for 1976 will only be voted in June 1975 no precise information is available at present The Board agreed upon the principle and entrusted the Organizing Committee with the task of making the necessary arrangements directly with Dr Petros Barvazian from WHO

Dr MacGregor expressed his willingness to seek financial means for making possible the participation in the Congress for a maximum of five speakers who have been invited by the Scientific Program Committee and who have no funds for making the trip to Mexico

9 Journal of FIGO

Dr Kaminetzky (USA) Chief Editor of the International Journal of Gynaecology and Obstetrics was unable to attend the Board meeting but his report had been circulated among the Board members prior to the meetings The agreement between FIGO and the new publisher Almqvist & Wiksell (Stockholm) was approved

Unfortunately Dr Ingelman Sundberg (Stockholm) could not obtain the publisher's agreement for bulk subscriptions at a lower subscription rate

The Secretary General pointed out that in its present form the Journal is not of any great use for FIGO as no income coming from the Journal can be expected in the near future and as the Journal contains very little material relating to FIGO's activities It is not likely that the number of paying subscribers will soon exceed 1500 the more so as the Journal is rather duplicating already existing national journals instead of developing features of its own and promoting the idea of international exchange of information

The Secretary General expressed his deep regrets that the abstracts of the last Executive Board meetings have not been published in the Journal

Dr Kaminetzky's suggestions of having special issues of the Journal on a precise subject prepared by a guest-editor invited for this task was well received by the Board These

special issues should contain contributions written on invitation by experts in a given field and representing various geographic areas

Dr Taylor put forward the idea that the Journal could mainly be devoted to a particular aspect of our specialty which is not dealt with by other journals. As an example Dr Taylor quoted social aspects in teaching and practice of Obstetrics and Gynecology.

The Board members felt that it was mandatory to appoint an editor at the place where the Journal is published in order to facilitate continuous and close contacts between the publisher and the Editorial Board. Therefore the Executive Board elected unanimously Dr Ingelman Sundberg Associate Editor of the Journal.

Taking up an idea put forward by the Norwegian Society of Obstetrics and Gynecology the Secretary General was asked to prepare newsletters on FIGO's activities such as abstracts of Board meetings, special committees activities etc. which should be circulated among the affiliated Societies with the request that they inform their members and possibly publish part of the newsletters in their own journals. The wish was expressed that this material of information should also be sent directly by the Secretariat to important journals as it is already done at present (French, Mexican, Argentine, Indian journals).

10 Cancer Committee of FIGO

Dr Ulfelder (USA) Chairman of the Committee was prevented from attending the meeting and had sent a report containing the following important points:

Volume 16 of the Annual Reports will be published and distributed prior to the VIIIth World Congress in October 1976.

Results of treatment for cases of cancer of the cervix, corpus vagina and ovary treated through 1968 will be reported in this volume.

The Cancer Committee has reviewed the staging method for cancer of the ovary and has approved on some changes which will be put before the General Assembly 1976 for ratification.

Minor changes in the staging instructions for cancer of the cervix have been elaborated by the Committee and will be dispatched to all collaborators in the Annual Reports. They will also be presented to the General Assembly in Mexico City.

The former project of staging for cancer of the vulva has been reviewed by the Cancer Committee and no changes were recommended. Therefore the General Assembly in Mexico City will be asked to ratify it and results of treatment for cancer of the vulva will be included in Volume 17 of the Annual Reports.

The Cancer Committee of FIGO has maintained contacts with the International Society for the Study of Vulvar Disease and the International Society for the Study of Trophoblastic Disease.

The Committee has also maintained links with the Commission on clinical oncology of the International Union Against Cancer (UICC), the American Joint Commission on Staging and End Results reporting the Committee on Oncology of the ACOG and with other bodies concerned with gynecological cancer.

It is hoped that a joint meeting of representatives of these working committees may take place around the time of the VIIIth World Congress in order to arrive at a consensus towards a solution of the minor differences in staging procedures which now exist.

The members of the Cancer Committee are: Dr L. A. Arrighi (Argentina), Dr J. Bonte (Belgium), Dr I. Hashimoto (Japan), Dr H. Husslein (Austria), Dr J. Holstad (Norway)—Vice-Chairman, Editorial Committee of the Annual Reports; Dr H. L. Kotrinc (Sweden)—Chairman, Editorial Committee of the Annual Reports; Dr K. A. McGarity (Australia)—Vice-Chairman, Cancer Committee; Dr J. H. Maus (Canada), Dr I. D. Neitchayeva (USSR), Mr R. B. Rickford (Great Britain), Dr H. Ulfelder (USA)—Chairman, Cancer Committee; Dr J. P. Wolff (France).

11 Committee on Qualifications

Dr Hubinont (Belgium) Chairman of the Committee was prevented from attending the meeting and unfortunately had not sent a report.

However the Executive Board discussed at length the organization of a Seminar devoted to Post-Graduate Training in Obstetrics and Gynecology to be held on the occasion of the VIIIth World Congress in Mexico City.

Dr Ratnam (Singapore) accepted to be the President of the Seminar and to organize it in cooperation with the local Organizing Committee and more especially with MacGregor, Secretary General of the Congress.

The Executive Board agreed upon the following preliminary schedule for this Seminar:

The Seminar will take place on Sunday, October 17, 1976, one session to be held before the Opening Ceremony and the second one afterwards.

The Societies affiliated with FIGO will be invited to send delegates to the Seminar.

The Seminar will be limited to the discussion of post graduate training and the main themes will be:

- 1) the training of the specialist should cover Gynecology as well as Obstetrics;
- 2) specific requirements for the training of such a specialist should be established and the qualification of a specialist be recognized by a diploma;
- 3) the participants in the meeting should express their opinion on the minimal and the optimal duration of training for obtaining this diploma;
- 4) a point of view should be expressed on the methods of training taking into consideration the time allotted for practical and/or theoretical training;
- 5) how should the level of knowledge and the ability of the trainee in the practice of Gynecology and Obstetrics be controlled? Examinations? Evaluation of the trainee's performances (work) by his monitors?
- 6) the value of refresher-courses for the specialist after they have obtained their diplomas.

It is hoped that the participants in the meeting will make recommendations concerning the various points mentioned above. These recommendations will be submitted to the General Assembly after having been accepted by this gathering. The resolution of FIGO would be addressed to all those concerned with training specialists in Obstetrics and Gynecology such as the

At present the pamphlet is being translated from English into the other languages. The final and definite manuscript should be ready by the end of this year. The Committee and its central office in Mexico are doing any effort to have this pamphlet ready for distribution and sale for the 1976 World Congress.

In the meantime the Committee is taking care of any request for information on Maternal Mortality Studies and is sending adequate material printed in mimeograph (duplicator) in English and Spanish.

The Committee has assisted the setting up of Maternal Mortality Studies Committees in Colombia and Costa Rica.

The World Survey on Maternal Mortality Studies is going on with success. Fortunately the prestige of the FIGO Committee has increased and now the Committee is receiving spontaneous communications on the statistical data of diverse committees.

15 Committee on Perinatal Mortality

The Secretary General announced that Dr Tesaro (Italy) Chairman of the Committee has recently undergone a surgical treatment and though being convalescent he could not attend the meeting.

Dr de Wateville recalled that the present objective of the Perinatal Mortality Committee is the collection of data for the preparation of annual reports on perinatal mortality. This publication would include uniform statistics on perinatal mortality received from big obstetrical departments as well as national statistics if available. This problem was discussed and it was felt that the Committee should request the cooperation of the World Health Organization and the Committee on Annual Reports and Definition of Terms in Human Reproduction (see page 10 of this document) which could greatly help the Perinatal Mortality Committee in the realization of this project.

The Board decided to appoint a Vice Chairman of the Committee with responsibilities. Dr Kloosterman (Netherlands) accepted this post.

16 Committee on Medical Aspects of Human Reproduction

Dr Taylor (USA) Chairman of the Committee commented on his report which had previously been sent to the members present.

The principal work of the Committee during the last year has continued to be the completion of the teacher's Manual on Reproductive Physiology, Elementary Demography and Family Planning.

The final manuscript consists of fifteen chapters or lectures with a total length of about 600 manuscript pages accompanied by 450 illustrations. The present plan is to have two so-called editions. The first or teacher's edition will have a loose leaf format and its illustrations will be duplicated by a set of lantern slides. Of this edition there will be 1500 copies published, one half with slides. The other edition perhaps properly called the student's edition will be firmly bound, still in three volumes but without lantern slides. Of the student's edition there may be 5000 copies printed and of course at a much lower cost.

A grant has been obtained to provide free of teacher's edition with slides to Departments of Obstetrics and Gynecology in developing countries that signal interest and intent to use the Manual. The student edition however will be for sale by the Massachusetts Institute of Technology Press at the low estimated \$6.00 or \$6.50 for each of the three volumes of the set.

In his report Dr Taylor furthermore proposed teaching seminars be organized in various parts of the world with selected members of the Committee present. The idea seems especially promising now as seminars would include a consideration of the Manual. The presentation of sets to departmental representatives.

Dr Purandare (India) was appointed Vice-Chairman of the Committee.

17 Joint Committee for the Study of Gynecological Problems in Childhood and Adolescence

Dr Rauramo (Finland) substituting for Dr Condon (France) Chairman of the Committee presented a report on the activities of the Joint Committee.

The Committee is presently organizing an International Symposium devoted to the Study of Gynecological Problems in Childhood and Adolescence to be held in Lausanne (Switzerland) from March 25-27, 1976. The main themes of the Symposium are: puberty of the female, contraception and pregnancy in the adolescent, and surgical treatment of genital malformation. There will be a program of free communications.

In his comments Dr Rauramo explained that contacts should be established with the World Health Organization. As an example a report from the WHO consultation on Pregnancy and Abortion in Adolescence in the process of being finalized. A close cooperation with WHO would be very useful to the Joint Committee. The Study of Gynecological Problems in Childhood and Adolescence and it would be easier to arrange a meeting of the Committee as a workshop together with WHO. Should the results of the workshop be interesting enough it might also be possible to publish a WHO book on the workshop because the questions of gynecological problems in childhood and adolescence are of an international interest.

Furthermore the Joint Committee is trying to establish closer contacts with pediatricians.

The Board appointed Dr Rauramo new Chairman of the Joint Committee in replacement of Dr Condon, nominated Honorary Chairman.

The new Chairman was entrusted with the task of recruiting members for his Committee which should be composed of gynecologists and pediatricians since pediatricians are starting a new Association both in USA and Europe for the study of adolescence in general.

Finally the name of Dr Dewhurst (Great Britain) suggested as the Vice Chairman of the Committee.

18 Varia

The International Federation received invitations to send a representative to the following meetings:

(a) XVIIIth International Congress of Obstetrics and Gynecology (Lausanne) June 21-28, 1975, co-organized by the International Confederation of Midwives.

omkinson (Great Britain) Chairman of the Joint Study Group on the Training and Practice of Midwives and Lactation Nurses agreed to be F I G O 's representative on this occasion

(b) World Conference of the International Women's Year Mexico City (Mexico) June 19-June 27 1975—organized by the United Nations in New York Dr MacGregor (Mexico) accepted to represent F I G O at this Conference

(c) International Conference for the Ninth Revision of the International Classification of Disease Geneva

(Switzerland) September 30-October 6 1975—convened by the World Health Organization Dr Ingelman Sundberg (Sweden) agreed to participate in these meetings as F I G O 's representative

19 Next executive Board meeting

The next Board meeting of F I G O will take place in Mexico City on Saturday October 16th 1976 the day prior to the Opening Ceremony of the VIIIth World Congress of Obstetrics and Gynecology

INFORMATION RECEIVED BY F I G O ON THE FOLLOWING CONGRESSES

Fifth European Congress of Perinatal Medicine Uppsala Sweden June 9-12 1976 Themes Socio-economic aspects of perinatal medicine breech delivery diabetes and pregnancy continuous monitoring of mother fetus and newborn infant For information contact the Secretariat of the Congress P O Box 1 S 750 14 Uppsala 14 Sweden

15th National Scientific Congress of The Israel Society of Obstetrics and Gynecology Tel Aviv Israel June 22-25 1976 Themes Infections in Obstetrics and Gynecology surgical trends in gynecology medical and surgical complications in pregnancy Free communications Sym-

posium on gynecological oncology For further information contact Professor R Toaff c/o Organizing Committee of the Congress P O Box 16771 Tel Aviv Israel

Fifth International Congress of Endocrinology Hamburg German Federal Republic July 18-24 1976 The Scientific Program includes plenary sessions symposia sessions and short communications In addition there will be workshops and special sessions For information contact The Congress Organizer Congress Reisebüro Hamburg Fifth International Congress of Endocrinology D-2000 Hamburg 36 Marseiller Strasse German Federal Republic

INCREASING CAESAREAN SECTION RATE

H E Johnell H Östberg and T Wahlstrand

*From the Department of Obstetrics and Gynaecology (Head Prof Carl A Gemell)
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tract At the University Hospital in Uppsala 719 caesarean sections (CS) were performed 1966-1970. The total number of deliveries was 16708. The CS rate increased from 7.0% to 7.8%. This trend was mainly due to an increasing frequency of CS performed for cephalopelvic disproportion, fetal distress, bad obstetrical history and failed induction of labour with intravenous tocin drip. No maternal deaths occurred. The neonatal mortality was 5.0%. In infants with a birth weight of 1500 g or less the perinatal mortality was 57% and in infants with a birth weight of more than 2500 g and gestational age of 37 weeks or more it was 0.9%. In 107 live CS on healthy mothers at term no perinatal deaths were noted.

During the late 1960s a remarkable increase of the caesarean section rate was observed in many Departments of Obstetrics and Gynaecology in Sweden (3,5). The aim of the present investigation was to study variations in the indications for CS in connection with a marked increase in the CS rate at a Swedish University Hospital and to study the influence of the increase on maternal and fetal mortality and morbidity.

MATERIAL

719 CS performed at the University Hospital in Uppsala during 1966-1970 were studied (Table I). This material is a Regional Hospital. The present series might be regarded as representative for a Swedish Central City Hospital with the exception that 20 CS were performed on Rh immunized mothers referred to the hospital from other districts.

METHODS

Records of the patients delivered by CS were reviewed with reference to maternal age, obstetrical history, indications for CS, operative technique, operation and post-operative complications, anaesthetic methods. Apgar score recorded one minute after birth.

birth weight, gestational age, perinatal mortality and neonatal morbidity. Only the main indication was recorded in each case, even if other indications were stated in the record. An X-ray pelvimetry was made in all cases delivered by CS for cephalopelvic disproportion. The capacity of the pelvic outlet was judged from the sum of the interspinous diameter, the intertuberous diameter and the sagittal outlet diameter (1,2). Patients with a true conjugate of the pelvic inlet of less than 9.5 cm or a sum of the outlet diameters of less than 29.5 cm were considered to have a contracted pelvis. Patients with a true conjugate of the pelvic inlet of 9.5-11.0 cm or a sum of the outlet diameters of 29.5-31.5 cm were classified as borderline cases (4). Previous operations on the uterus, i.e. classical CS, myomectomy or Strassman's operation were classified as uterine scars. Indications such as multipara without living child, long standing infertility, multiple spontaneous abortions and previous complicated deliveries were called bad obstetrical history. Abnormal fetal presentations were sub-divided into three groups: 1) breech presentation in elderly primipara, 2) transverse lie and 3) other malpresentations. Breech presentation was always combined with other indications such as elderly primipara, uterine inertia, cephalopelvic disproportion, diabetes or fetal distress. These cases were recorded as the indication combined with breech presentation. Lower segment incisions, transverse or longitudinal as well as classical incisions were used. Hysterectomy was performed only for special indications. In some cases sterilization was carried out by division of the Fallopian tubes.

All patients were anaesthetized by a trained anaesthetist. 0.5 mg of atropine was used as premedication. The basic method of anaesthesia was induction with nitrous oxide-oxygen and pentothal sodium. An endotracheal intubation was performed with succinylcholine relaxation, which was the method of muscle relaxation until the delivery of the fetus and the placenta. The patient was ventilated with nitrous oxide-oxygen 1:1 until the delivery. The anaesthesia was then continued with artificial ventilation with nitrous oxide-oxygen 3:1 with curare relaxation and the analgesia was completed by pethidine or in a few cases halothane, methoxyfluorane or diethyl-ether. In 15 cases the pentothal sodium was replaced by propofol and in 17 cases diethyl-ether was added during the induction. In

Table 1 Incidence of Caesarean Sections (C S) and perinatal mortality for infants delivered by C S at the University Hospital in Uppsala 1966-1970

Year	Deliveries (n)	C S (n)	C S (%)	Perinatal mortality (%)
1966	3 409	68	2.0	11.8
1967	3 450	104	3.0	2.9
1968	3 509	122	3.5	7.3
1969	3 217	181	5.6	7.7
1970	3 123	244	7.8	4.4
1966-1970	16 708	719	4.3	5.0

one case of eclampsia nitrous oxide and halothane were used for the anaesthesia as barbiturates and pethidine in high doses were used in the treatment of the patient prior to the C S.

Body temperature of more than 37.0°C in the morning or of more than 37.5°C in the afternoon after the third post-operative day in cases treated with antibiotics was recorded as puerperal fever if no specific explanation was found in the record.

The perinatal mortality was defined as the sum of the number of stillborn infants with a gestational age of more than 28 weeks and the number of infants who died within 7 days of birth.

All infants were immediately taken care of by a paediatrician who estimated the Apgar score one minute after birth. Infants with an Apgar score of 7-10 were considered vigorous, those with 4-6 slightly depressed and those with 1-3 severely depressed.

The infants were classified according to Yerushalmy (9). The 5 groups recommended by him are defined in Table V.

All infants delivered by C S were observed in the Department of Paediatrics at least 24 hours after the delivery. The causes of perinatal deaths were confirmed by post-mortem examinations in all cases. In infants with neonatal morbidity the diagnosis was made by a paediatrician. Minor congenital malformations and slight neonatal hyperbilirubinaemia were not taken into consideration.

In order to estimate the risk for the infant at C S special attention was paid to a group of elective C S performed on healthy mothers at term.

RESULTS

The C S rate increased about 4 times during the period under investigation (Table 1) while the number of deliveries was fairly constant. Altogether 683 women were operated on: 649 once, 32 twice and 2 three times. No maternal deaths occurred after C S or after vaginal deliveries during this period.

The distribution into different age groups was fairly constant during the period of investigation.

The maternal age at C S was on an average 35 years. 55% of the women operated on were primigravidae. A slight increase in the frequency of primigravidae was seen during the last 2 years. The number of women with three or more previous deliveries decreased from about 9% in 1966 to 6% in 1970. The relative frequency of patients with previous abortions, C S or operative vaginal delivery was fairly constant. As a comparison to the given it can be mentioned that the mean age at delivery was 26.3 years in 7190 deliveries at the Department during 1967-1969 (7). At the Uppsala Hospital Region 47.4% of the women were primigravidae out of 96753 deliveries, 1964-1968 (8).

Cephalopelvic disproportion was the most common indication (Table II). This indication was about 4 times in frequency. Forty patients had contracted pelvis, 229 had a border line pelvis, 11 had a normal pelvis according to the definition. The frequency of all three types of pelvis was higher at the end of the period than at the beginning. Among patients delivered by C S for cephalopelvic disproportion fetal distress and bad obstetrical history were in 1970 about 10 times more frequent than in 1966 as indications for C S. Psychological indications were noted only the last two years. Immunization and transverse lie were most common during the last year. Many indications for placenta praevia, abruptio placentae and other malpresentations and prolonged labour showed no upward trend toward the end period.

Addition of indications were stated in about a fifth of the cases without any significant increase during the period.

It was noted that attempts to induce labour with intravenous oxytocin drip were given an increasing frequency and C S performed more often. Thus emergency C S were performed in 15% of fetal distress occurring in connection with oxytocin drips once in 1966 and 12 times in 1970. The increasing number of cases (5, 11, 13, 18, 25 respectively) C S was made when an oxytocin drip and in some cases artificial rupture of membranes failed to induce labour. These were recorded under the indication 'failure of induction of labour'.

Lower segment transverse incisions were made 683 times and lower segment longitudinal 23 times. Classical incisions were made 7 times for

ble II Indications for Caesarean Sections at the University Hospital in Uppsala 1966-1970

	1966	1967	1968	1969	1970	1966-1970
cephalopelvic disproportion	??	38	57	79	84	280
maternal distress	5	11	14	37	47	109
obstetrical history	7	2	5	17	19	45
placenta praevia	4	14	17	3	6	39
ruptio placentae	3	8	10	6	17	39
diabetes mellitus	9	6	5	8	9	37
immunization	4	4	6	3	17	29
other malpresentations	7	5	2	5	7	26
leukaemia	1	3	2	5	6	17
transverse lie	2		1	4	8	15
primigravida	3	1		6	4	14
clamped umbilical cord	1		4	4	7	11
uterine inertia	1	1	1	2	5	10
uterine scar			1	3	3	7
obstetrical indication				1	5	6
immaturity				1	3	4
rigid cervix	1	1			1	3
uterine malformation					3	3
previous vaginal plastic surgery		1			7	3
obstetrical presentation in						
early primigravida		2				2
inert uterine rupture	1			1		2
still disease		1	1			2
obstructed birth canal		1				1
cellaneous	2	4		1	6	13

cations such as transverse lie, placenta praevia, cervical cancer. Hysterectomy was performed once for atonic bleeding, once for placenta praevia and once for bilateral bleeding from the uterine arteries. Injuries to the urinary bladder occurred 6 times and an intestinal injury once. Although 103 patients were operated on previously, 1 C.S. a dehiscence of an old uterine scar was noted only once. Bleeding exceeding 600 ml was noted in 164 cases. In 32 of these cases bleeding occurred prior to the C.S. Sterilization was combined with C.S. 10 times.

In one case a serious complication of anaesthesia occurred. After the injection of 250 mg pentothal sodium and 75 mg succinylcholine bronchospasm occurred together with an acute laryngeal oedema, spasm of the vocal cords, which was not relieved by additional succinylcholine. Treatment consisted of 100 mg hydrocortisone and 0.3 mg adrenaline given with prompt effect. The patient was delivered under local anaesthesia of the abdominal wall, nitrous oxide-oxygen inhalation. The infant was vigorous with an Apgar score of 8. The uncomplicated post-operative course was noted in 581 (81%) of the mothers. Puerperal fever occurred 45 times (6.3%), endometritis 25 times

(3.5%) and urinary infections 13 times (1.8%). Wound complications occurred 42 times (5.8%). Venous thrombosis was seen once. No pulmonary embolism occurred (Table III).

The number of infants was 726, twins occurred 7 times. The infants were vigorous at birth in 86%, slightly depressed in 6% and severely depressed in 8% (Table IV). Infants with a low one minute Apgar score had a considerably higher neonatal mortality than other infants. All neonatal deaths occurred in cases anaesthetized with the basic method of anaesthesia. In cases with variations of the basic method, i.e. adding diethyl ether during the induction

Table III Maternal complications after delivery by Caesarean Section at the University Hospital in Uppsala 1966-1970

Puerperal fever	45
Wound complications	42
Endometritis	25
Urinary infections	13
Bronchopneumonia	4
Paralytic ileus	3
Pneumothorax	2
Eclampsia after delivery	1
Venous thrombosis	1
Miscellaneous	5

Table IV Neonatal deaths in relation to Apgar score one minute after birth by Caesarean Section at the University Hospital in Uppsala 1966-1970

Apgar score	Infants (n)	Neonatal deaths	
		n	%
7-10	670	9	1.5
4-6	42	6	14
1-3	56	13	23

tion of anaesthesia or replacing pentothal sodium by propanidid the frequency of low Apgar scores did not differ from the total series

The number of stillborn infants was 8 (Table V). In 5 of these cases the infant was thought to be alive immediately before the C S. In 3 cases the fetus was known to be dead but the C S was performed for another important indication. The total perinatal mortality was 5.0%. It was higher in the first year than during the rest of the period. The perinatal mortality was highest in Group I and lowest in Group V. If the infants were sub divided only according to birth weight the perinatal mortality was 24% in Groups I-III and 2.1% in Groups IV-V.

The perinatal mortality in all deliveries at the Department during the same period was 1.9% (0.7% for infants with a birth weight of more than 2500 g and 2.7% for infants with a birth weight of 2500 g or less).

The most common cause of neonatal death after delivery by C S was idiopathic respiratory distress syndrome (IRDS) (Table VI). In 5 of the 13 deaths in IRDS erythroblastosis was also present. No infants in Group V died from IRDS. Congenital

malformations were equally distributed over Groups. Cerebral haemorrhage was only seen in Group V. In the case of placental infection, membranes ruptured 7 days before the C S.

Neonatal morbidity was diagnosed in 11 (16.5%) of the live born infants.

The number of elective C S performed in healthy mothers at term was 207 (29%). The number of elective C S increased more than the total C S rate. Twins occurred once. Two infants had a birth weight of less than 2500 g. Most of the infants were vigorous at birth but 6 were slightly and 1 severely depressed. Neonatal morbidity was seen in 11 infants (5%) but all survived the neonatal period. IRDS was diagnosed in 2 infants, aspiration pneumonia in 2 infants, hypoglycaemia in 2 infants and pulmonary atelectasis in 1 infant. One of the twins had difficulties in maintaining a normal body temperature. Congenital hydrocephalus in one infant was diagnosed prior to the C S by X ray.

DISCUSSION

The incidence of C S for different indications in the present investigation is compared with the results of the previous study (6) in Table VII. It is evident that the number of C S performed for placental indications, toxæmia, transverse lie, old primigravida, incompetent uterine rupture and heart disease were unchanged. The frequency of these indications was also stable during the period of the present investigation. This was also true for the indications abruptio placentae, diabetes mellitus and malpresentations, but these indications were more common in the present investigation than

Table V Stillborn infants and neonatal deaths in connection with Caesarean Sections at the University Hospital in Uppsala 1966-1970

The infants were classified according to Yerushalmiy (9)

Infant group	Birth weight (g)	Gestational age (weeks)	Infants (n)	Stillborn (n)	Neonatal deaths (n)	Perinatal mortality (%)
I	1500 or less	All ages	7	1	3	57
II	1501-2500	Less than 37	68	2	13	27
III	1501-2500	37 or more	20	2	2	20
IV	2501 or more	Less than 37	72	1	7	11
V	2501 or more	37 or more	559	2	3	0.9
I-III	2500 or less	All ages	95	5	18	24
IV-V	2501 or more	All ages	631	3	10	2.1
I-V	All weights	All ages	726	8	28	5.0

vious one. As regards uterine scar breech presentation in elderly primigravida and obstructed third canal a decrease was seen.

The entity 'bad obstetrical history' as defined in the present investigation may be widened to include 1) primigravida breech presentation in elderly primigravida and uterine scar. In that case an increase from 31.2 per 10000 deliveries during the 1950s (6) to 40.7 in the present investigation was seen. In fact the incidence of such cases was lower during the first 3 years of the present investigation and higher during the last 2 years.

The indications that were most responsible for increased C.S. rate were cephalopelvic disproportion, fetal distress and bad obstetrical history. An increase was also noted in the number of patients who had been exposed to attempts to induce labour prior to the C.S. Three causes may be responsible for these changes. Firstly increased attention to the possibility of cephalopelvic disproportion and to the previous history during the prenatal period; secondly more active obstetrical supervision; increased attention to deviations from the normal progress of labour and signs of fetal distress; thirdly a tendency to give up an attempt to induce labour when there was no response to one or two intravenous oxytocin drips and in some cases artificial rupture of the membranes. The situation may be summarized as an attempt to reduce the risks of delivery. The perinatal mortality in Sweden is low. The death of an infant during delivery is regarded as a disaster. Thus C.S. are performed with increasing frequency in order to avoid complications in labour.

Table VI Causes of neonatal deaths after caesarean Section at the University Hospital in Uppsala 1966-1970

Infants were classified according to Yerushalmy (9). Definition of the infant groups see Table V.

	Infant groups					
	I	II	III	IV	V	I-V
Chronic respiratory distress syndrome	1	6	1	5		13
Neonatal thrombocytopenia		5		1		6
Neonatal haemorrhage		1	1	1	1	4
Neonatal infection	2				2	2
Neonatal trauma		1				1

Table VII The incidence of Caesarean Section for some indications per 10000 deliveries in the County of Kopparberg 1951-1960 (6) and at the University Hospital in Uppsala 1966-1970 (present investigation)

	County of Kopparberg 1951-1960	Present investigation
Cephalopelvic disproportion	57.6	167.6
Fetal distress	3.6	65.2
Bad obstetrical history	5.3	76.9
Placenta praevia	21.8	73.3
Abruptio placentae	5.0	23.3
Diabetes mellitus	10.3	22.1
Other malpresentations	4.4	15.6
Toxaemia	11.7	10.4
Transverse lie	8.1	9.0
Old primigravida	11.7	8.4
Prolapsed umbilical cord	0.5	6.6
Uterine inertia	3.8	6.0
Uterine scar	8.9	4.2
Psychiatric indication		3.6
Breech presentation in elderly primigravida	5.3	1.2
Imminent uterine rupture	1.7	1.2
Heart disease	0.5	1.2
Obstructed birth canal	3.7	0.6

The perinatal mortality at C.S. was lower in the present investigation than during the 1950s in the County of Kopparberg (6). In the latter study the results at the Central County Hospital (Group A) were better than in the whole County and more comparable to those of the present investigation. In A in the first series all mothers with Rh immunization were excluded. If the same procedure were made in the present investigation the perinatal mortality would be 3.6% (1.7% for infants with a birth weight of more than 2500 g and 1.9% for infants with a birth weight of 2500 g or less). Thus the perinatal mortality was obviously lower in the present investigation. This may to some extent be due to wider indications for C.S. but also to improved prenatal care of the mothers and neonatal care of the infants.

The group of elective C.S. performed on healthy mothers at term may mirror the risk for the infant at delivery by C.S. No perinatal mortality was noted and the neonatal morbidity was lower than in the total series. However neonatal morbidity was observed in 5% of these infants. The most serious complications was IRDS in 2 cases. The risk for an infant may be lower at a normal vaginal delivery than at a C.S. But who can guarantee to a pregnant

woman that her delivery will be a perfectly normal one?

Was the increasing C/S rate justified? It is very difficult to give an answer on the basis of the present study. The intention with almost every C/S was to secure not only a living but also a healthy infant. Furuhyelm (5) studied C/S in cases of fetal distress. She followed up the babies through child health centres. Such a follow up might be the best way to solve this problem which was outside the aim of the present study.

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DISTRIBUTION OF BLOOD FLOW IN MYOMATOUS UTERI AS MEASURED BY LOCALLY INJECTED $^{133}\text{XENON}$

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Abstract. Regional blood flow in the myometrium and myomata has been measured during laparotomy in 11 patients using locally injected $^{133}\text{Xenon}$. Blood flow was calculated from the wash-out curves using both initial peak technique and compartment analysis. Assuming equal partition coefficients in myometrium and myomata, following values in ml/min/100 g are seen: initial slope technique—myometrium 15.6 ± 2.79 , myomata 5.1 ± 0.95 ; compartment analysis—myometrium 0.1 ± 0.21 , myomata 5.5 ± 1.16 . The blood flow was always lower in myomata than in myometrium of the same uterus. The main reason for the reduction of blood flow per unit weight of uterus in patients with myomata previously reported thus seems to be low blood flow in the myomata. A slight reduction of blood flow in the surrounding myometrium, however, also seems probable.

observations cited above (7-10) may depend on a generalized slowing of blood flow in the myomatous uterus as a whole, blood flow in myomata and the surrounding myometrium being of about the same magnitude. They may also be the result of a very low blood flow in myomata and only a slight or no reduction in the perfusion of the surrounding myometrium. Previous studies do not give any information on blood flow within myomata as compared with normal myometrium in the same uterus. The present work was intended to compare within the same uterus and at the same occasion blood flow in myomata and in the intervening myometrium.

In a previous study (7) using the isotope clearance method, it was shown that blood flow per unit weight of myomatous uterus is reduced in comparison with non myomatous uterus. Similar conclusions can be drawn from a study by Klingenberg (8). This is believed to be the result of an impaired blood flow within the myomata. Clinical experience is in agreement with this idea. Degenerative changes in uterine myomata are frequent and are thought to reflect insufficient blood supply. How far morphological data indicate great variations in vascularity in myomata (3, 4, 8, 13). Furthermore, the blood vessels of the surrounding myometrium are affected by the myomata. This was described by Sampson (14) and in a more recent study by Farrer Brown et al. (2). By compressing veins myomata may impair drainage of the surrounding myometrium and endometrium. This may lead to pathological uterine bleeding. It also leads to a reduction in blood flow in normal myometrium surrounding myomata. Thus the

METHODS AND MATERIAL

The isotope clearance method utilizing $^{133}\text{Xenon}$ was used. The theoretical details have been described in previous papers (5, 6, 17). The injection technique has been that described by Jansson (9). All measurements were made during laparotomy with the uterus exposed. 0.1-0.15 ml of a solution of $^{133}\text{Xenon}$ dissolved in 0.9% NaCl solution was injected via the uterine serosa. In a few cases a smaller volume was used—identical volumes injected into both myomata and myometrium. In myomata the injection depth varied with the size of the tumor, but in all cases the isotope was deposited well inside the capsule of the myoma. The injections into normal myometrium were made at a depth of 0.5 to 0.7 cm from the serosa. The needle was as a rule held obliquely to the surface to ensure a needle track of at least 0.7 to 1 cm. The injection needle had an outer diameter of 0.4 mm. No aspiration was made prior to isotope injection. Injection time was 20 sec and the needle was left in situ for another 20 sec in order to minimize the risk of leakage through the needle track. Recording was carried out as described for intraarterial injections (5). In all cases at least two recordings were carried out. Since remaining isotope from previous injections might interfere with following recordings, old

isotope depots were shielded with lead so that they were not seen by the detector

Two types of curve analysis were performed. The initial slope method for curve analysis was applied on all curves using the initial portion of the curve but disregarding any steep fall of the curve immediately after removal of the isotope syringe and needle from the visual field of the detector. Compartment analysis was performed when the elimination curve was of the multiexponential type. In order to calculate total blood flow of the labelled region the compartments were weighed together by taking the percentage of activity at the end of the injection as judged by extrapolating curve components to time +70 sec. This percentage is a measure of the relative size of compartments within the labelled tissue volume. Regional uterine blood flow (RUBF) then has been calculated according to eq. 1

$$\text{RUBF} = \frac{A_1 F_1 + A_2 F_2}{100} \quad (1)$$

where A_1 and A_2 stand for percent radioactivity at time +20 sec in compartments 1 and 2. F_1 and F_2 are blood flows in compartments 1 and 2. The validity of this type of curve analysis has been discussed in a previous paper (6). In all calculations of blood flows the partition coefficient of skeletal muscle 0.7 (1) was used.

The patients were undergoing radical or conservative surgery because of myomata. Anaesthesia was of the routine type excluding halothane. A semi-open non-rebreathing system was used to prevent rebreathing of isotope. Blood pressure was within normal limits during the operation. The cases are presented together with results in Table I.

Statistical analysis was carried out using a pairing design test (16).

RESULTS

In 11 patients a total of 25 measurements were carried out. From normal myometrium only out of 11 curves were of the mono-exponential type. The rest were multiexponential curves.

In 14 recordings from myomata 13 were of the monoexponential type and only 1 multiexponential. Curve analysis according to the initial slope method (Table I) gave as a rule lower values than the compartment analysis (Table II). A statistical analysis of values according to the initial slope method showed a statistically significant difference was seen between normal myometrium and myomata ($p < 0.001$). If curves are analyzed according to the compartment method even greater differences result. Myomata always had lesser perfusion than normal myometrium. Mean blood flow \pm standard error of the mean in myomata was 5.1 ± 0.95 ml min⁻¹ 100 g⁻¹ and in myometrium 15.6 ± 2.29 ml min⁻¹ 100 g⁻¹ when calculated from the initial slope of the curve. Compartment analysis is carried out the values for myomata 5.5 ± 1.16 and for myometrium 20.0 ± 3.21 ml min⁻¹ 100 g. No correlation between myoma size and blood flow was seen. Neither was there any correlation between blood flow in myomata and normal myometrium in same uterus. Two myomata had a multi-exponential wash out curve. One of them was in fact

Table I Blood flow (ml min⁻¹ 100 g⁻¹) in myometrium and myomata according to initial slope

Pat	Age	Phase of cycle	RUBF myometrium	Type of curve	Size of myoma	RUBF myoma	Type of curve	Remarks
F E	46	Prolif	16.7	Multiexp	3 cm	11.0	Monoexp	
G A	43	-	34.7	Multiexp	5 cm	0.5	Monoexp	
A E	31	Prolif / secr	70.2	Multiexp	7 cm	5.1	Monoexp	Small volume of isotope
L S	40	Secr	14.3	Multiexp	3 cm	1.0	Monoexp	Small volume of isotope
P O	47	-	15.6	Multiexp	7 cm	8.9	Monoexp	
W B	51	-	6.9	Monoexp	5 cm	8.5	Monoexp	
					1 cm	1.8	Monoexp	Small volume of isotope
					5 cm	3.8	Monoexp	
S I	78	Prolif	10.8	Multiexp	8 cm	4.4	Multiexp	Adenomyoma
J I	46	-	9.7	Multiexp	5 cm	0.6	Monoexp	Pelvic varicose
J R	48	Secr	6.2	Monoexp	6 cm	5.6	Monoexp	
L A-G	44	Secr	21.1	Multiexp	10 cm	11.8	Multiexp	
I B	49	Prolif	15.6	Multiexp	4 cm	4.4	Monoexp	
Mean			15.6			5.1		
S.E.M.			7.79			0.75		

Table II Blood flow ($\text{ml min}^{-1} 100 \text{ g}^{-1}$) according to compartment analysis in cases with multiexponential curves

at	Measured region	Quick component	Slow component	~ quick component	RUBF
E	Myometrium	53.9	7.6	45.7	28.8
A	Myometrium	44.1	17.3	81.8	39.2
E	Myometrium	53.9	14.9	39.3	30.2
S	Myometrium	24.3	9.7	48.9	16.6
O	Myometrium	25.6	10.2	49.8	17.7
I	Myometrium	19.4	8.5	17.2	10.4
I	Adenomyoma	19.4	0.8	29.0	5.4
I	Myometrium	78.5	7.7	27.1	13.3
A-G	Myometrium	44.1	13.1	65.5	33.4
A-G	Myoma 10 cm	30.3	7.1	41.5	16.7
B	Myometrium	22.1	8.8	64.6	17.4

enomyoma. Both belonged to the largest tumors in the series. Several myomata showed slight degenerative changes according to the pathologist's report but no case of necrotic myoma was included.

DISCUSSION

It is clearly shown that myomata in this study have significantly lower blood flow than normal myometrium in the same uterus. This seems to be in agreement with clinical observations and morphological data. The main reason for the low uterine blood flow in myomatous uteri in the studies by Klingenberg (10) and Forssman (7) thus seems to be low blood flow within the myomata. From the present study the effect of myomata on the perfusion of surrounding myometrium cannot be evaluated. The recorded blood flow values for myometrium are low compared to those of Jansson (8). For a reliable comparison, however, it would be necessary to have groups that were comparable with respect to age, parity, hormonal status and technique of isotope injection. Such factors do not interfere with the conclusions of the present study. For each patient is her own control as we compare blood flow in myometrium and myomata. Blood flow in myomata seems to be homogeneous in most cases. In only two out of 14 measurements was the wash-out curve of the multiexponential type. Furthermore, one of these tumors was not a simple myoma but an adenomyoma. However, the fact that myomata can show a multiexponential elimination curve indicates that there

may exist well and less well perfused compartments within the same tumor. When the well perfused portion is of very small weight as compared with the less well perfused the graphical separation of curve components becomes difficult. The same applies when perfusion rates are of similar magnitude. Thus it may be that different components are not discovered by the present type of curve analysis. It must also be stressed that the only thing really measured is the elimination of isotope from the labelled part of the tissue. If a small non representative volume of tissue is labelled false conclusions may be drawn from the resulting wash-out curve when results are extrapolated to the whole organ or tissue. The present study very distinctly points out one potential risk in the use of the isotope clearance method for the study of uterine blood flow. This is the risk of injecting isotope into a myoma not representative for the bulk of uterine tissue. When injections are made under the control of the eye and the palpating hand during laparotomy this risk is small. When injections are made via the uterine cavity (5, 12, 15) or via the vaginal fornices (11) this danger is increased. By taking special care in cases with myomata and by making repeated injections with different positions of the injection needle this risk would seem to be diminished.

The partition coefficient has been taken to be 0.7 which holds true for most non fatty tissues. If major variations occur between myomata and myometrium the conclusions concerning differences in blood flow may not hold true. This factor clearly needs to be controlled.

The present study also gives some information on

the problem of the anatomical counterparts of the components of the elimination curve after local and intra arterial injections of isotope. In most cases in this study myometrium had two clearly separated components. Most myomata had one component that was not identical with either of the other two. This would theoretically result in a three component curve after intra arterial injections of isotope. A few three-component curves were seen after intra arterial injections in myomatous uteri. In these cases however the first component was of a magnitude never seen in the present study. The obvious conclusion is that the two components usually calculated are the weighed means of families of similar perfusion rates. This does not invalidate the calculations of RUBF based on the compartment analysis but the calculations of relative weights of tissue corresponding to the different blood flows becomes unimportant.

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A PROSPECTIVE STUDY OF DRUGS AND PREGNANCY

II Anti emetic drugs

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RESULTS

Spontaneous and induced abortion

Fig. 1 compares the use of anti-emetic drugs during the different pregnancy months in four groups of women: women who will have a miscarriage (417), women who will have an induced abortion (158), women who will give birth to a dead infant (133), and women who will give birth to a live infant (5620). The curve for women who will later miscarry obviously lies below the others, especially during months III-IV. Women who will later have an induced abortion do not differ from women who will have a live infant. In order to evaluate the statistical significance of these differences, the use of anti-emetic drugs during the third month was compared between women who will have a living normal infant—591 used anti-emetics out of 4934 women (11.9%)—and women who will later have a miscarriage—22 used anti-emetics out of 423 (5.2%). A χ^2 test gives $\chi^2=17.5$ at 1 d.f. $P<0.001$.

Antiemetics are nearly exclusively used because of morning sickness. Such complaints were reported in 212 of 423 women who will abort (50%) and in 3645 of the 4934 women who will have a living normal child (74%). The reduced frequency of morning sickness in women who will later miscarry may be part of an explanation to the low intake of anti-emetics. A comparison of the groups using the frequencies of 22/212 (10.4%) and 591/3645 (16.2%) instead gives $\chi^2=5.3$ at 1 d.f. $0.05>P>0.02$.

Further details of the association between various factors and the presence of morning sickness

Abstract In a prospective study of anti-emetic drug use during 6376 pregnancies, the previously demonstrated gave correlation between the use of such drugs and miscarriage was confirmed. It is probably completely due to less frequent and possibly less severe morning sickness. No correlation is found between the use of anti-emetics and infant malformation. A detailed analysis showed that this was mainly due to a high incidence of drug use (mainly promethazine) among women giving birth to infants with congenital dislocation of the hip. The probable indirect relationship is discussed.

The first paper in the present series (5) factors associated with the use of psychopharmaca during pregnancy were discussed, based on a prospective study of drug use in pregnant women in Malmö during 1963-65. The present paper reports a similar study of anti-emetic drugs (mainly histamines) based on the same material. The possible hazards of antihistamine drugs have been briefly discussed. They are used mainly during early pregnancy when morning sickness is common and organogenesis takes place, and they have been used in a vast number of pregnant women. Soon after the thalidomide tragedy, suspicions were raised about some drugs with anti-emetic properties, especially meclizine (9). The general opinion now is that no teratogenic effects can be demonstrated, but a recent study on diphenhydramine by Saxen (7) reopened the question.

MATERIAL AND METHODS

A detailed description of the material and methods used in this study is referred to the first paper of this series (5).

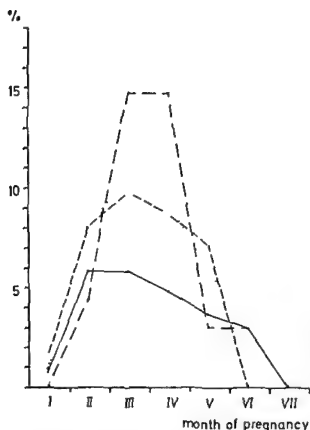


Fig 1 Percentage of women using anti-emetic drugs during different months of pregnancy according to pregnancy outcome — = miscarriage - = induced abortion =live infant =dead infant

the use of anti-emetics are given in Table I. The mean age of women who do not report morning sickness is significantly higher than that of women with morning sickness ($t=3.1$ $P<0.001$) but there is no significant difference between those who have morning sickness but do not use anti-emetics and those who use such drugs ($t=1.1$ N.S.)

The percentage of wanted pregnancies is significantly lower among women not having morning

sickness than among women who report this complaint ($\chi^2=54.6$ $P<0.001$). There is however no difference from this point of view between drug users and non users ($\chi^2=2.3$ N.S.) but the trend is toward a higher incidence of wanted pregnancies in the former group. This would fit with a correlation between desire for pregnancy and morning sickness. A similar trend is of course also seen in the percentage of women married at LMP—increasing from the group reporting no vomiting to a maximum in the group using anti-emetic drugs ($\chi^2=11.2$ d.f. $P<0.001$).

In order to study whether these factors explain the difference in the frequency of morning sickness between women who miscarry and women who have a child, these two groups were split according to civil status (married at LMP or not) and desire for pregnancy. Table II gives the results. The difference in the frequency of morning sickness between the two groups of women is consistent in all groups but the effect of desire for pregnancy within civil status is apparent and the effect of civil status within the groups of wanted or unwanted pregnancies is also seen but is perhaps less clear.

Apparently pregnancies which end in miscarriage are less prone to morning sickness than pregnancies which result in the birth of a child. Perhaps also those women in the former group who do report morning sickness have less severe complaints than such women in the latter group. This could explain the remaining underrepresentation of anti-emetics in the group of potential miscarriages. Another possible explanation is that civil status and/or desire for pregnancy influence the time of first contact with a gynecologist. This problem is analysed in Table III for women who will bear a live normal child. It can be seen from this Table that both factors act together—an increased frequency of early visits (during the first trimester)

Table I Some characteristics associated with morning sickness and use of anti-emetics

	No morning sickness	Morning sickness but no anti-emetics	Anti-emetics
No. of women	1 797	3 705	894
Mean age of women with S.E.M.	26.85±0.17	26.14±0.08	26.38±0.17
% wanted pregnancies	76.1	83.6	85.7
% married at L.M.P.	58.8	67.6	68.8

Table II Morning sickness, civil status and desire for pregnancy. All women who gave birth to a live normal infant or who had a miscarriage

	% of women reporting morning sickness			
	Married at L M P		Not married at L M P	
	Wanted pregnancy	Not wanted pregnancy	Wanted pregnancy	Not wanted pregnancy
Miscarriage	56.1	45.8	5.9	36.8
Giving normal child	75.1	69.6	72.8	70.9

en in the most positive group—married at L M P with a wanted pregnancy. This group will therefore have a slightly increased chance of having anti-emetics prescribed and this could be one cause of the higher intake of anti-emetics in pregnancies which continue to term than in pregnancies ending in miscarriage.

Antenatal death and malformations

Fig. 1 there is an indication that women who will give birth to a dead infant used more anti-emetics especially during the third and fourth month of pregnancy than women who will have a live infant. Fig. 2 shows a similar tendency for women who will give birth to infants with major malformations while the group with infants with minor malformations agrees actively well with the normal group. Fig. 3 compares the best group—normal live infants—with the total group of perinatal pathology including both infants with major malformations and infants which die before the age of one. The difference is not noticeable. Table IV shows use of anti-emetics during the 1st trimester in different categories. Again a surplus of anti-emetic usage can be found in the group

of major malformations but it does not reach statistical significance ($\chi^2=1.9$ at 1 d.f.). At least part of this explanation can be that morning sickness is more common among women who will have a malformed infant ($178/200=89\%$) than among women who will have a normal infant ($3680/5002=73.6\%$). This difference is statistically significant ($\chi^2=23.9$ at 1 d.f. $P<0.001$). The percentage of women who have morning sickness but do not use anti-emetic drugs is thus also increased in the major malformations group (72% compared with 60% in the control group $\chi^2=81.2$ at 1 d.f. $P<0.001$).

Another approach was made in comparing the use of anti-emetics and the birth of malformed or dead infants. 194 infants with major malformations and 194 matched controls were compared (for an account of the method of matching see (5)) and 93 infants who died before one year of age (53 were stillborn) were compared with 93 matched controls. The results are shown in Table V. Among the mothers of dead infants the use of anti-emetics was in this sample even lower than among the controls but the tendency to increased anti-emetic use among women who had malformed infants remained and even reaches statistical significance.

Table III Time of first contact with a gynaecologist, civil status and desire for pregnancy among women who gave birth to a normal live infant

Status	Desire pregnancy	No. of women with first visit to a gynaecologist	
		During 1st trimester	After 1st trimester
Married at L M P	Wanted	806 (85%)	2070
	Not wanted	49 (7.7%)	188
Not married at L M P	Wanted	767 (71.0%)	986
	Not wanted	94 (9%)	37

6.9 at 3 d.f. $P<0.001$

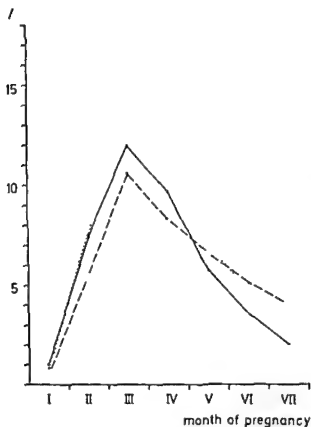


Fig. 2. Percentage of women using anti-emetic drugs during different months of gestation. Comparison between normal infants (—) infants with minor (---) and infants with major (· · ·) malformations.

χ^2 test: $0.025 > P > 0.01$. Morning sickness was more common in the malformation group (178 of 194) than among the controls (144 of 194).

The controls for the malformed infants, selected in this way, show a lower than expected incidence of drug usage when compared with the total group of women giving birth to normal live infants. The reason for this is not clear—a similar phenomenon was seen in the analysis of psychopharmaca usage and was discussed briefly then (5).

Table V also shows the use of anti-emetics among women who bore infants with certain types of malformations. The actual numbers are small but there is one group which shows a markedly high usage of anti-emetic drugs, namely women whose infants have congenital dislocation of the hip (CDH)—there are 37 such women and 13 of these used anti-emetic drugs (11 of them promethazine) during the first trimester instead of the 4 expected. Only 2 of 35 women who had infants with congenital heart disease had used anti-emetics.

Fig. 4 shows the incidence of women with morning sickness. Women with morning sickness but not using promethazine and women using promethazine, comparing three groups of infants: had CDH when they had other malformations and when they had no malformations at all. The high incidence of promethazine users in the first group is evident—the incidence is somewhat higher in the group of other malformations compared with controls, but this small difference becomes still more reduced when the incidence of drug users is referred to the number of women with morning sickness: 11% in the malformation group and 8.3% in the control group (16 of 12 of 144 resp.). This small difference is probably no more than random variation. It cannot be concluded, however, that the slightly raised incidence of 11% in the malformation group might be random variation below a truly significant level. At $P=0.05$ a doubling of the true malformation

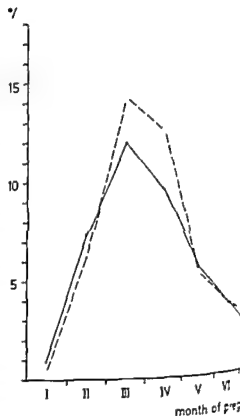


Fig. 3. Percentage of women using anti-emetic drugs during different months of pregnancy. Live normal infants (—) compared with the group of stillbirths (---) and infants with major malformations (· · ·).

Table IV Use of anti emetic drugs during the first trimester by women who had a normal infant or an infant with different kinds of malformations

ant gory	Total number	Number using anti emetics	Numbers using drugs containing			
			Pro- methazine	Prochlor perazine	Diphen hydramine	Meclozine
	5 753	778	617	91	46	19
mal	5 002 (87.0%)	679 (87.0%)	538	81	39	18
or malform	551 (8.6%)	65 (8.4%)	57	8	4	1
major	105 (1.7%)	23 (3.0%)	16	7	3	0
major malforma	95 (1.5%)	11 (1.4%)	11	0	0	0

nance can still be hidden. However, we conclude except for the congenital dislocations of the no correlation between drug use and malformation probably exists.

Infant length and birth weight

A difference is demonstrable in the duration of pregnancy (Table VI) or birth weight at term (Table VII) between pregnancies ending with the birth of a normal infant with and without anti-emetic drug use in the first trimester.

DISCUSSION

Women who are destined to have miscarriage—but not those that will have an induced abortion—use anti-emetic drugs less extensively than women who are going to give birth to a live infant. This is partly due to the fact that women who will miscarry experience morning sickness less frequently than women who will have an infant, as was also pointed out by Yerushalmi & Milkovich (10). When a correlation was made for the different frequency of

Table V Number of women using anti emetics in general and promethazine in particular during the first trimester. Infants with major malformations and matched controls: infants without malformation but dead in one year with matched controls

Expected numbers calculated from the incidence of anti-emetic users in the total group of women giving birth to normal infants (679/5 002) or from all controls.

I group	Total number	Number using anti-emetics	Expected no. from total group	Number using promethazine
malform	194	3	6.3	7
controls for major malform but not malformed	194	16	6.3	12
controls for dead but not malformed	93	10	1.6	7
controls for major malform and/or major malform	93	15	1.6	10
controls	87	47	38.9	34
	87	31	38.9	
<i>Specific malformation types</i>			<i>Expected no. from all controls</i>	
major malform	11	3	1.1	—
lip and/or palate	10	0	1.1	0
heart malform ± other malform	15	—	3.8	2
spina bifida	15	3	1.6	3
dysplasia of hip	37	13	4.0	11
pleural malform	10	0	1.1	0
Down	10	2	1.1	1

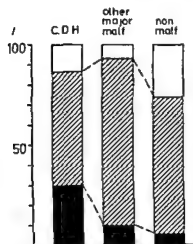


Fig. 4 Percentage of women using anti-emetics (black columns) with morning sickness but not using anti-emetics (hatched columns) and without morning sickness (empty columns). Women whose infants had congenital dysplasia of the hip (CDH) compared with women whose infants had other major malformations and with women who had normal infants.

morning sickness a slight and just statistically significant difference was still left. It could be claimed that this difference is also an artefact and due to differences in social factors or perhaps to differences not only in the incidence of morning sickness but also in the severity of the complaint when present.

The discussion has helped to clarify an apparent protective effect of the drugs. That such an effect would really exist is perhaps unlikely. Similar considerations should be applied when an apparent harmful effect of a drug exists. This can be exemplified by the correlation between the use of anti-emetic drugs and the birth of infants with major malformations. Previously, Villumsen (1970) also

found a higher consumption of anti-emetic women who go on to have a malformed infant, compared with women who have a normal infant, whereas the opposite was found by A. Forfar (6).

One explanation could be that morning sickness occurs more often in pregnancies ending in the birth of a malformed infant than in normal pregnancies. Doring & Hossfeld (4) claimed that women with hyperemesis gravidarum run an increased risk of having a malformed infant. In the study it was shown that there is an increase in drug users but also in women having morning sickness but not using drugs when the infants with major malformations is compared with the group of normal infants.

A more detailed analysis shows that anti-emetic drugs—mainly promethazine—were mainly used in one group of women, namely those who had an infant with congenital dislocation of the hip. The cause of this abnormality is probably a congenital one, an important factor being the endocrine disturbance during the pregnancy (1). It may be that genes are also implicated. However, it is hard to understand how a drug administered during the first trimester could cause this abnormality. A likely explanation would be that the endocrine disturbance causing the CDH is also somehow associated with more severe vomiting and therefore with a higher likelihood of drug usage. The incidence of morning sickness is not increased in the CDH group compared with the group of other major malformations (32 instead of 33 expected) but the effect could be more severe.

According to textbooks, morning sickness and vomiting is more prevalent in conditions with

Table VI Duration of pregnancy when anti-emetic drugs were and were not used during the first trimester. Normal infants according to sexes

	Total number	Less than 38 weeks	38-42 weeks	More than 42 weeks	χ^2 (d.f.)
<i>Boys</i>					
With drug	340	79	302	9	0.9 N.S.
Without drug	649	15	1806	91	
<i>Girls</i>					
With drug	339	7	307	10	0.7 N.S.
Without drug	2035	139	183	67	

χ^2 between drug groups within sexes: 3.1 at 4 d.f. N.S.

N.S. = non significant

Table VII Birth weight among normal infants when the woman had or had not used anti emetic drugs in the first trimester

	Total number	Birth weight kg						χ^2 (5 d.f.)
		< 3.5	3.5-3	3-3.5	3.5-4	4-4.5	> 4.5	
With drug	340	7	40	106	114	64	9	8.1 N.S.
Without drug	7049	87	709	674	778	797	69	
With drug	339	9	49	177	114	35	6	7.4 N.S.
Without drug	7038	95	330	801	617	156	39	

Between drug groups within sexes = 15% at 10 d.f. N.S.
= non significant

gonadotropin (HCG) level such as hydatidiform mole and twins. HCG levels are often low in women in the time preceding a miscarriage (3) which would fit with the observation that vomiting is infrequent during pregnancies ending with a miscarriage. CDH is six times more frequent in girls than in boys (1). A significant correlation has been found between sex of the conceptus and the blood gonadotropin level of the mother (3) with a higher level when the fetus is female. Such an indirect mechanism is one possible explanation of the finding of increased promethazine use in women who later have an infant with CDH.

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THE INTRAVENOUS INFUSION OF PROSTAGLANDIN $F_{2\alpha}$ IN THE MANAGEMENT OF INTRAUTERINE DEATH OF THE FETUS

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Prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) was given intravenously in the treatment of 16 cases of intrauterine death. Delivery was achieved without complications or effects in 15 out of the 16 patients. A posterior cervical incision in one patient treated with cervical dilatation and $PGF_{2\alpha}$ infusion.

Safe methods are required for the induction of labour. For medical and biological reasons delivery is desirable as soon as the diagnosis is made. In this study it is shown that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) can be used for the induction of labour in cases of fetal death in the third trimester of pregnancy.

PATIENTS AND METHODS

Seventeen patients were carrying a dead fetus after 196 or more days of pregnancy. The estimated mean duration of intrauterine death was 5.9 days with a range from 1 to 14 days. In most cases amniotomy was done primarily. $PGF_{2\alpha}$ was administered as a solution in normal saline by a constant pump infusion through a cannula in a forearm. $PGF_{2\alpha}$ was given at an initial rate of 40 mcg/minute increased by hourly increments until a maximum dose of 80 mcg/minute.

RESULTS

In 15 out of the 16 patients were delivered successfully. The average induction-delivery interval was 7 hours and 36 minutes (Table I). Complications occurred in none of the cases. Except for a local reaction in the infused vein side effects such as nausea, vomiting, diarrhoea or pyrexia were not observed. There was one case of failed induction. At the start of infusion vaginal examination revealed an elongated firm cervix. Løvset's cervical

instrument was used for mechanical dilatation of the canal. $PGF_{2\alpha}$ infusion produced strong contractions. After 10 hours vaginal examination revealed an unchanged cervix and the infusion was stopped. Amniotomy was performed the following day and Hartel's dilators were used together with $PGF_{2\alpha}$ for 40 hours. Pelvic examinations then revealed a completely effaced cervix with a 2 cm rigid external cervical os. A short time after a fetus was found emerging from the vagina. Inspection revealed an intact cervical os and a transverse posterior cervical laceration. The placenta was delivered through the laceration. A repair was carried out vaginally. The patient was given antibiotics and no postoperative complications occurred.

DISCUSSION

There is good evidence for the value of the prostaglandins as agents for induction of labour in cases of intrauterine death. The infusion of prostaglandin E_2 (PGE_2) has been reported by Kanm (4) and by Filshie (2). In these series the mean infusion-delivery intervals were 12 or 8 hours respectively but side effects such as nausea, vomiting and diarrhoea were common.

A simultaneous intravenous infusion of PGE_2 and oxytocin seems also to be effective and the incidence of gastro-intestinal side effects is reduced (5). Another valuable alternative in the treatment of intrauterine death is the extra-amniotic administration of the prostaglandins $F_{2\alpha}$ or E_2 (1).

The present study suggests that the intravenous infusion of $PGF_{2\alpha}$ for the induction of labour in cases of intrauterine death is a valuable method. In 15 out of 16 cases the induction was successful. The mean infusion-delivery interval was 7 hours and 36

Table 1 Prostaglandin $F_{2\alpha}$ intravenous infusion in 15 successful cases with intrauterine death of the fetus

	Mean \pm S D	Range
Maternity (days)	238 \pm 28 20	196–303
Prostaglandin $F_{2\alpha}$ total dose (mg)	20.3 \pm 11.80	2.7–44.4
Infusion delivery interval (hours minutes)	7.36 \pm 4.09	1.15–15.45

minutes and gastro intestinal side effects were avoided

In one case of unsuccessful induction a posterior cervical rupture occurred in association with the infusion of $PGF_{2\alpha}$. Similar accidents are reported following intra amniotic administration of $PGF_{2\alpha}$ for mid trimester abortion (3, 6, 7). In our case the infusion had lasted for 50 hours and should have been abandoned earlier.

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A NEW TECHNIQUE FOR THE LOCALIZATION OF THE CERVIX AT ^{113m}In PLACENTAL SCINTIGRAPHY

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Abstract. Sixty women in the third trimester of pregnancy examined with ^{113m}In placental scintigraphy because of suspected placenta praevia. In 4 of the cases the scintigraphic procedure was initiated by application of a Meyers ring containing ^{113m}In around the cervix. In the other cases the fundus and symphysis pubis were marked by means of a small radioactive source. Complete placenta praevia was correctly detected in 8 cases and marginal placenta in 4 cases. Scintigraphic localization of the placenta was correct in 10 women where the ring was used, whereas when the usual marker was used seven errors occurred. The authors therefore recommend this new technique in order to facilitate the evaluation of the scintigrams.

Localization of placenta in cases of antepartum hemorrhage can be accomplished with different methods e.g. plain film radiography, thermography, amniography and scanning with isotopes. Ultrasound Among these isotope and ultrasound methods are the safest and most reliable. Drukker (1) obtained a correct diagnosis in 26 of 28 patients by manual counting after the intravenous administration of radio iodinated serum albumin. Use of a gammacamera shortens the procedure thus minimizing the risk of caval syndrome. Recently ^{113m}In (^{113m}In) has been introduced as an excellent marking agent for placenta localization and experience with this method has been reported by several authors (2, 3, 4, 5). This study presents the results of ^{113m}In placental scintigraphies including a new method of cervical marking carried out during a 10 year period (1972-73) in our departments.

MATERIAL AND METHODS

Sixty women in the third trimester of pregnancy were examined with ^{113m}In placental scintigraphy. The women all suspected cases of placenta praevia because of

bleeding. In 24 of the cases the scintigraphic procedure was initiated by application of Meyers ring containing $100\text{ }\mu\text{Ci}$ ^{113m}In around the cervix in order to mark precisely this important anatomical region (Fig. 1). Before placing the intravaginal marker $50\text{ }\mu\text{Ci}$ ^{113m}In was placed in each of the two holes in the solid plastic ring. In no case has the application and removal of the ring caused bleeding or other complications. In 36 cases the fundus and the symphysis pubis were marked by means of a small radioactive source.

^{113m}In was eluted from a ^{115}Sn generator with dilute HCl (0.1 N). The raw eluate was used and 1 mCi of ^{113m}In was administered intravenously. Scintigraphy was carried out 10-15 minutes after injection. The women were examined by means of a Pho/Gamma HP gammacamera. Anterior and both lateral views were collected on Polaroid films. A total of about 50 000 counts was obtained for each view using a high energy (410 KeV) diverging collimator and with a discriminator set to accept photons around 393 KeV.

^{113m}In has a physical half life of 1.7 hour and emits a single

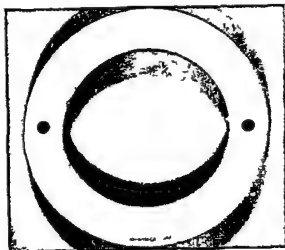


Fig. 1 Meyer's ring. In each of the two holes $50\text{ }\mu\text{Ci}$ ^{113m}In is applied and the holes are closed with a strip.

Table I Results of ^{113}In In placenta scintigraphy in 60 patients

No of patients	Technique for marking		Scintigraphy	Final diagnosis
	Pubic symphysis	Meyers ring		
8	4	4	Complete placenta praevia	Complete placenta praevia caesarean
1	1		Marginal placenta	Complete placenta praevia caesarean
4	2	2	Marginal placenta	Marginal placenta caesarean section
1	1		No placenta praevia	Marginal placenta caesarean section
1	1		Complete placenta praevia	Normal attachment of placenta vaginal d
4	4		Marginal placenta	Normal attachment of placenta vaginal
41	23	18	No placenta praevia	Normal attachment of placenta vaginal d
60	36	24		

gamma ray of 393 keV. When injected intravenously ^{113m}In is associated with transferrin and this complex circulates throughout the blood including the placenta with very little transfer across the placenta to the fetus. The biological half life of ^{113m}In in the blood has been found to be 6.7 hour (2). The absorbed dose received from 1 mCi ^{113m}In to the total maternal body has been calculated to 17 mrad to maternal blood 146 mrad and fetal blood 8 mrad (2).

RESULTS

Table I shows the results of ^{113m}In In placental scintigraphy compared with diagnosis in 60 women. The final diagnoses were obtained by vaginal deliv-

ery or by Caesarean section. Complete praevia was correctly detected in 8 cases (Fig. 1), marginal placenta in 4 cases. One complete praevia was interpreted as marginal placenta; a marginal placenta was diagnosed as not placenta praevia by scintigraphy (2 false negative). In 41 of normal placenta the scintigrams were interpreted as complete placenta praevia in one case and marginal placenta in 4 cases (5 false positive). The remaining 41 cases were all correctly diagnosed as normal.

In the two false negative and the five false positive cases the fundus and the symphysis pubis were marked by means of a small radioactive s-

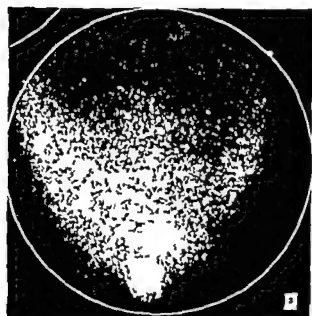


Fig. 2 A 25 year-old pregnant woman submitted because of vaginal bleeding. Meyer's ring is used for marking. (a) Anterior view. Most of the activity is located low in the

pelvis. (b) Left view. Accumulation is seen low anteriorly. Caesarean section three weeks later revealed a complete placenta praevia.

DISCUSSION

The results of this study confirm that ^{113m}In scintigraphy may visualize the placenta correctly as reported by other authors (2, 3, 4, 5). However, there were 7 mistakes in the isotopic localization of the placenta. In these cases the fundus and the symphysis pubis were marked by means of a small radioactive source so that the relation of the placenta to the internal cervical os was difficult to interpret. When a radioactive ring was used to indicate the cervix, all the scintigraphic diagnoses of placenta which were checked were correct. Therefore we recommend this new technique in order to facilitate the evaluation of scintigrams.

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3. A 27-year-old woman in the 37th week of gestation. In this case the symphysis pubis was marked. Left lateral view shows accumulation low in the pelvis. Above and below is a round area with less activity, indicating the position of the fetus. Sixteen days after scintigraphy a caesarean section was carried out. A boy lying in transverse position with the head to the left was delivered and a complete placenta previa was removed.

In the radioactive ring round the cervix was used, the scintigrams were correctly interpreted in all cases compared with the final diagnosis. In some cases ^{113m}In scintigraphy gave unexpected information about the fetus. Fig. 3 illustrates such a

INFLUENCE OF AGING UPON THE URINARY HORMONE EXCRETION IN THE MALE

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Abstract The urinary excretion of LH low polar steroids neutral C₁₉ and C₂₁ steroids was measured in 100 healthy males aged 20-79 years. No significant changes could be noted for the excretion of LH, pregnadiol and 17-ketogenic steroid between 50 and 79 years. The excretion of androsterone and aetiocholanolone significantly lower in the oldest group (70-79 years). A significant drop in the excretion of DHA was noted at over 60 years. The excretion of low polar oestrogens remained constant from 20 to 55-59 years but showed a highly significant ($P < 0.001$) decrease at about 60 years. This drop might reflect a sudden decrease in the plasma levels of oestrone sulphate due to a decreased sulphuryl activity in the liver.

production, metabolism and excretion of steroid hormones in the male has been extensively studied and usually there is a fair good correlation between blood levels and urinary excretion. On the other hand the oestrogens in males have been considerably less studied. One main reason for this might be the difficulties involved in measurement of low levels of oestrogens in biological fluids. Most methods used for routine urinary oestrogen determinations are neither specific nor sensitive to be applied to urine from normal males. Routine estimations of oestrogens in urine were not available until recently but the introduction of radioimmuno assay and competitive protein binding had caused a dramatic change in this field.

Conflicting results have been presented about the influence of aging upon the urinary excretion and the blood levels of oestrogens in the male. A few studies have been carried out and their results are summarized in Tables I and II. While Behrsohn & Fife (5) found an increased urinary excretion of

oestrone, oestradiol 17 β and oestriol, Pincus (7,8) found a slight continuous decrease in the excretion of oestrone + oestradiol 17 β and an almost constant excretion of oestriol. It should be mentioned that the results of Pincus were in agreement with those of an earlier bioassay investigation carried out by that author (27). Kaufmann (22) noted no significant change in the excretion of oestrone and oestradiol 17 β . The studies on blood hitherto reported indicate increased levels of unconjugated oestrone and oestradiol 17 β with increasing age (15, 25, 31).

As one part of a study on the influence of aging upon the production and metabolism of steroid hormones in the male we have measured the urinary excretion of low polar oestrogens (oestrone + oestradiol 17 β), neutral C₁₉ and C₂₁ steroids and LH in normal healthy males aged 20-79 years. One part of the results dealing with the low polar oestrogen excretion has been presented in a previous communication (35).

ABBREVIATIONS AND TRIVIAL NAMES

Aetiocholanone 3 α -hydroxy 5 β -androstan-17-one
Androsterone 3 α -hydroxy 5 α -androstan-17-one
DHA 3 β -hydroxy 5 α -androstan-17-one
DHA sulphate 3 β -sulphoxy 5 α -androstan-17-one
epiandrosterone 3 β -hydroxy 5 α -androstan-17-one
LH luteinizing hormone (synonym ICSH)
oestradiol 17 β 13 β 5 (10)-oestratriene-3 17 β -diol
oestriol 13 β 5 (10)-oestratriene-3 16 α 17 β -triol
Oestrone 3-hydroxy 13 β 5 (10)-oestratriene-17-one
oestrone sulphate 3-sulphoxy 13 β 5 (10)-oestratriene-17-one
pregnanediol 5 β -pregnane-3 α 20 α -diol
testosterone 17 β -hydroxy-4-androsten-3-one

Table I Results from various studies on the age dependence of the urinary excretion of oestrogens in normal males

Age (y)	n	Urinary excretion in $\mu\text{g}/24 \text{ h}$ mean (range)				Ref
		Oestrone	Oestradiol 17 β	Oestrone+ oestradiol 17 β	Oestrol	
20-48	21	4.3 (1.7-9.8)	1.1 (0.3-1)	5.4	2.6 (0.6-9.9)	3
45-65	20	6.3 (2.8-12.5)	2.1 (0.5-3.8)	8.4	6.0 (1.3-17.7)	3
25-39	45			3.48	4.77	13
40-44	83			3.12	4.78	13
45-49	101			2.84	4.19	13
50-59	56			2.63	4.46	13
60-71	23			1.98	4.13	13
21-75	8	7.41	3.52	10.93		13
26-30	9	6.59	3.44	10.03		13
31-35	7	5.48	4.08	9.56		13
36-40	4	4.07	2.75	6.82		13
41-50	15	6.52	4.01	10.53		13
51-60	18	4.59	2.94	7.53		13
61-70	11	5.52	3.30	8.82		13
71-83	6	5.49	3.38	8.87		13

MATERIAL

The younger groups consisted of 41 healthy males aged 20-49 years. Most of them were fathers of newly born babies delivered at our Department and the others had normal spermograms. The older groups consisted of 61 healthy men aged 50-79 years. They had been admitted to hospital for minor operations predominantly for hernia and varices. There was no evidence of endocrinological disorder or abnormalities in the prostate in any case. Values for haemoglobin, Na, K, sedimentation rate, urinary residual nitrogen and urinary sediment were normal and all patients were free from medications. 24 hour urine samples were collected preoperatively.

METHODS

LH was determined by the radioimmunosorbent technique of Wide and co-workers (39, 40) and was expressed as IU of the 2nd International Reference HMG preparation/24h.

Table II Results from various studies on the age dependence of the plasma levels of unconjugated oestrogens in normal males

Age (y)	n	Mean plasma levels in pg/ml		Ref
		Oestrone	Oestradiol 17 β	
22-61	50		16.6	15
67-90	34		25.6	15
21-40	20	30.1	26.5	25
70-80	10	43.0	21.8	25
<50	30	43	14	31
>65	30	57	21	31

Low polar oestrogens were determined by the method of Carlström & Furuholm (13). Fractionated 11-deoxy ketosteroids (DHA, androsterone and aetiocholanediol) and pregnanediol were determined by a combination of methods of Carlström et al. and Carlström & Friberg (11, 12). 17 ketogenic steroids were assayed as described by Birke et al. (6) and total 17 ketosteroids by the method of Vestergaard (38).

RESULTS

The results are given in Tables III and IV. There was no significant change in the excretion of LH with age, although the mean value for the oldest group was higher than for the two other groups. Low polar oestrogen excretion was constant from 20 to 50 years. At about 60 years this excretion was significantly decreased with about 40% (highly significant, $p < 0.001$). The excretion of DHA decreased significantly ($p < 0.1$) at about 60 years. Values for androsterone, aetiocholanediol and fractionated 11-deoxy 17 ketosteroids and total ketosteroids were probably significantly decreased in the oldest group ($p < 0.1$). No significant differences were noted for 17 ketogenic steroids and pregnanediol although the mean values for pregnanediol were lower in the two older groups.

DISCUSSION

No significant age-dependent change in the excretion of LH was found in the present study.

ble III Influence of aging upon the urinary excretion of conjugated low polar oestrogens estrone+oestradiol 17 β in normal males

e)	n	Low polar oestrogens $\mu\text{g}/24$ hrs	
		Mean	Range
29	0	5.2 \pm 0.4	3-8
39	16	5.0 \pm 0.4	3-7
49	5	4.6 \pm 0.3	2-7
59	24	5.4 \pm 0.5	2-11
69	26	3.3 \pm 0.3	1-7
79	13	3.1 \pm 0.4	1-6

n agreement with the results of Wide and co-workers (40). However, an age-dependent increase in LH levels has been reported by other workers (31-34).

Androsterone and aetiocholanolone are mainly excreted as glucosiduronates (for a review see 7). The age-dependent decrease in the urinary excretion of these steroids has previously been demonstrated by other workers (4, 28). The age-course of the urinary excretion of androsterone+aetiocholanolone is similar to that of the urinary excretion of conjugated testosterone and to the plasma levels of unconjugated testosterone (36, 37). Approximately 50% of the urinary androsterone and aetiocholanolone has been shown to be derived from testicular precursors (21).

The main part of the urinary DHA in the male is of testicular origin, although a certain testicular production of this steroid can not be excluded (21). In the

plasma as well as in urine DHA is present almost exclusively as its sulphate (7). In this present study a probably significant decrease in the urinary excretion of DHA was noted at about 60 years, which is in agreement with previous findings by other authors (4, 28). A similar decrease in the plasma levels of DHA sulphate was demonstrated by Viikko (41). Viikko found that plasma levels of the sulphates of androsterone, epiandrosterone, 5 α -pregnen-3 β , 20 α -diol and DHA decreased abruptly at about 60 years in males and about ten years earlier in females. Thus the urinary excretion of DHA seems to reflect the plasma levels of this steroid quite well.

The excretion values obtained for pregnanediol were close to the specificity limit of the method used and the results should therefore be interpreted conservatively. Romanoff and co-workers (30) have reported an age-dependent decrease in the urinary pregnanediol excretion in males. In the present study the mean values for pregnanediol were lower in the two older groups, but this difference was not statistically significant. It should be mentioned that Adlercreutz and co-workers (2) found a close correlation between the urinary excretion of DHA and pregnanediol during normal menstrual cycles. They concluded that the excretion of these two urinary steroids might reflect the production of 3 β -hydroxy-5 α -pregnen-20-one, which is a proximate precursor to DHA as well as to progesterone/pregnanediol.

The excretion of low polar oestrogens in the present series remained constant from 20 to 55-59 years and thereafter decreased abruptly. These findings

Table IV Influence of aging upon the urinary excretion of LH and conjugated neutral C₁₉ and C₂₁ steroids in normal males

Androsterone E=aetiocholanolone 5 α /5 β ratio=ratio androsterone/aetiocholanolone 17 KS=17 ketosteroids 17 KGS=17 ketogenic steroids P diol=pregnanediol

	50-59 years			60-69 years			70-79 years		
	Mean	Range	n	Mean	Range	n	Mean	Range	n
U/24 hrs	38 \pm 3	17-63	18	37 \pm 4	8-74	24	48 \pm 7	21-95	10
mg/24 hrs	0.39 \pm 0.14	0.05-0.40	27	0.11 \pm 0.03	0.05-0.70	76	0.11 \pm 0.03	0.05-0.30	13
g/24 hrs	2.15 \pm 0.27	0.7-5.9	27	1.91 \pm 0.17	0.9-4.1	26	1.8 \pm 0.17	0.3-2.8	13
g/24 hrs	1.58 \pm 0.18	0.5-3.4	22	1.37 \pm 0.15	0.4-3.4	6	1.05 \pm 0.21	0.3-2.9	13
ratio	1.47 \pm 0.12	0.55-1.84	22	1.85 \pm 0.27	0.59-5.00	6	1.66 \pm 0.76	0.74-4.00	13
of DHA A and E	4.08 \pm 0.57	1.4-11.3	22	3.30 \pm 0.28	1.3-7.5	76	2.17 \pm 0.46	0.4-6.0	13
17 KS mg/24 hrs	6.75 \pm 0.54	3.1-10.7	0	6.13 \pm 0.43	2.7-13.0	76	4.77 \pm 0.41	2.9-8.1	13
GS mg/24 hrs	8.45 \pm 0.76	3.5-15.9	19	8.07 \pm 0.70	3.8-16.2	26	7.37 \pm 0.87	3.6-13.1	13
1 mg/24 hrs	0.21 \pm 0.04	0.05-0.60	19	0.14 \pm 0.03	0.05-0.60	76	0.14 \pm 0.04	0.05-0.40	17

support the results of Pincus (27-28) but are not consistent with those presented by Behrsohn & Oelefse and by Kaufmann (5-22). The results of the later authors might have been influenced by methodological factors since the excretion values presented are about twice as high as those given by Pincus and from the present study.

From several studies it is known that approximately 80% of the low polar oestrogen fraction in male urine consists of oestrone (5, 8, 16, 20, 24, 29). The sulphoconjugate of oestrone is indeed the most abundant low polar oestrogen in peripheral plasma as well as in the bile from males as well as from pregnant and non pregnant females (1, 9, 14, 19, 23). As far as we know from the literature no simultaneous assays of conjugated oestrogens in male urine and plasma have been carried out but it is known from studies on pregnant women that the urinary excretion reflects the plasma levels quite well (18). It might therefore be assumed that the drop in low polar oestrogen excretion at about 60 years might reflect a sudden decrease in plasma oestrone sulphate which in turn coincides with the previously demonstrated drop in plasma neutral steroid sulphate levels (41).

Vermeulen and co-workers (37) have recently studied the urinary excretion pattern of the metabolites of injected radioactive testosterone in young (<50 years) and old (>68 years) males. They found that the transformation into glucosiduronates remained constant and independent of age while the amount of sulpho-conjugated metabolites formed in the older group was only about 40% of that in the younger group. The decrease in urine and plasma values for steroid sulphates might therefore be attributed to a decrease in the sulphurylating activity in the liver. It has recently been demonstrated by Carlstedt Duke & Gustafsson (10) and by Eriksson (17) that *in vivo* administration of androgens to rats decreased the *in vitro* steroid sulphurylating activity in the liver while oestrogens increased this activity. It is therefore tempting to speculate over a regulatory effect of sex steroids on the sulphurylating activity in man. Thus it might be possible that an age-dependent drop in the oestrogen production causes a decrease in the liver sulphurylating activity.

A decreased sulphurylating of oestrone might lead to increased levels of unconjugated oestrone and oestradiol 17 β in plasma and this might explain the increased plasma levels of unconjugated oestrogens

found in older males (15, 25, 31). Oestradiol is generally considered as the most potent oestrogen and thus the increased levels of steroid might at first speak against an age-dependent decrease in sulphurylating activity. However, it should be kept in mind that the plasma levels of unconjugated oestrogens in males are approximately one tenth to one fifth of the oestrone sulphate levels (19, 23). Oestrone sulphate might exert considerable biological effects after its hydrolysis in the target organs (for a review on this subject see Adlercreutz (3)). Furthermore the plasma levels of unconjugated oestrogens in older males might be strongly influenced by increased metabolic clearance rate and increased levels of steroid binding proteins (31, 34). The estimation of unconjugated oestrogens in plasma might not give a true picture of the total oestrogen production in the male. We are therefore repeating this investigation using radioimmunoassay of oestrone sulphate in plasma and the results are published later.

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LUMBAR EPIDURAL ANALGESIA IN LABOUR

A Clinical Analysis

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Abstract Lumbar epidural block was given for pain relief to 296 women in labour. Bupivacaine was used as the analgesic agent and the technique is described in detail. Satisfactory analgesia was obtained in 97% of the cases during the first stage of labour and in 84% during the second stage of labour. The frequency of instrumental deliveries and of occipito-posterior position increased but the clinical condition of the newborn seemed to be unaffected.

Introduction Lumbar epidural analgesia in labour with bupivacaine as the local analgesic agent has been used since 1969 at the Department of Obstetrics and Gynaecology, Karolinska Hospital, Stockholm. This retrospective survey of 296 deliveries with epidural block was done to summarize our clinical experience with this type of analgesia. Epidural block during labour has been used for many years on the North American continent but as the experience varies in different countries, the experience of others cannot always be transferred. This report should be seen as a survey of epidural block during labour in a Swedish Hospital.

MATERIAL AND METHODS

Patients In the period July 1969 to March 1963 a total of 296 women (all vaginal deliveries) were given an epidural block during labour at the Department of Obstetrics, Karolinska Hospital. Their age ranged between 15 and 38 years (25 years). 252 were nulliparae and 44 multiparae. All others had uncomplicated pregnancies and all babies were singleton and born as vertex presentations.

Indication Epidural block was mainly given when unsatisfactory analgesia was obtained with conventional methods (nitrous oxide and morphine). Occasionally the indication

for this type of analgesia was an unusual fear of labour pains and in these cases labour was often induced and the patient was given an epidural block as soon as she had regular contractions. In patients with incoordinated uterine action the epidural block was performed both to regularize contractions and to alleviate pain (7).

Contraindications

Epidural block was not given to patients with central nervous system diseases, allergy to local analgesic agents and infections of the lumbar skin area. Patients with coagulation disorders were also excluded.

Borderline pelvis as evaluated by X-ray pelvimetry, breech presentation and multiple pregnancies were considered as obstetrical contraindications to epidural block.

Technique

The epidural space was entered with an 18 gauge Tuohy needle in the second or occasionally third lumbar interspace. A midline approach with the loss of resistance technique was used and a catheter was inserted through the needle for 4-5 cm in a cranial direction. The external portion of the catheter was secured to the patient's back with adhesive tape and the distal end connected to a bacterial filter (Millipore®) through which all injections were made. Aspiration through the catheter was done in all cases before injection of the test dose. In eight cases no catheter was used and only one single injection was given due to the late stage of labour.

In cases of unsatisfactory analgesia a more concentrated analgesic solution was used and if the patient was still uncomfortable the epidural catheter was re-

Table I Analgesia during the first and second stage of labour after epidural blockade

Labour	Satisfactory analgesia (%)	Unsatisfactory analgesia (%)
First stage	92	8
Second stage	84	16

Table II Lumbar epidural analgesia in labour all cases Frequency of spontaneous deliveries instrumental deliveries and caesarean sections

	No	Normal delivery		Instrumental delivery		Caesarean section	
		No	%	No	%	No	%
Nulliparae	757	130	51.6	106	47.1	16	6.3
Multiparae	44	37	84.1	6	13.6	1	2.3
Total	797	167	56.4	112	37.8	17	5.8

positioned Reposition of the catheter was done in 12 cases

The maternal blood pressure was recorded with a cuff manometer every 5–10 minutes during the first 30 minutes after each injection and then every 15 minutes Hypotension was defined as a fall in systolic blood pressure to a level below 100 mmHg In all cases an intravenous cannula was inserted and 500–1500 ml of 5% glucose was infused throughout labour In the last hundred cases the infusion was started 10–15 minutes before analgesia and 500 ml of fluid was rapidly infused to counteract blood pressure falls

Drug dosage

Bupivacaine 0.25% with adrenaline $1/100\,000$ (Marcaine Adrenaline[®]) was used as analgesic agent and administered at demand about every second hour in doses of 6–8 ml after an initial test dose of 2 ml In 53 cases a 0.5% solution was used to achieve adequate analgesia The mean total dose given was 44 mg (range 14–192 mg) and the mean number of injections given to each patient was 7.5 (range 1–9)

Control of patients

The epidural block was started when the patient was in need of pain relief had regular contractions and the cervix was open for more than 2–3 cm

Before analgesia was given the obstetrician evaluated the bituberous diameter and checked that the fetal head was fixed in the pelvic inlet X-ray pelvimetry was performed in all cases with uncertain pelvic capacity If the membranes had not ruptured spontaneously amniotomy was performed at a cervical dilatation of 4–5 cm The degree of bladder distension was checked regularly throughout labour Instrumental delivery was performed in all cases when the presenting part had been on the pelvic floor for more than 60 minutes

The fetal heart rate (FHR) was recorded by direct auscultation every 15 minutes during the first stage and every 3–5 minutes during the second stage of labour In 61 cases a cardiotocograph (Hewlett Packard no 8070 A) was used for continuous recording of FHR and uterine contractions

Evaluation of the analgesic effect

The analgesic effect of the blockade was evaluated by the anaesthetist during the first and second stage of labour

and registered on a protocol The analgesia was *satisfactory* when the patient did not groan seemed to be pain free and comfortable All were judged as *unsatisfactory*

RESULT

Analgesic effect

In 24 cases (8%) the analgesia was judged *satisfactory* during the first as well as the second stage of labour (Table I) In two further cases the analgesia was judged *unsatisfactory* because the space could not be localized and no block was performed In 46 cases (16 per cent) the analgesia was judged *satisfactory* during the first stage of labour and *unsatisfactory* during the second stage In 23 of these cases a pudendal block (10+10 ml 1% Prilocaine) was given for the second stage

Complications

A dural puncture occurred in 3 cases In these cases this complication was recognized before the anaesthetic solution was injected In one case an epidural block was performed without complications through another lumbar interspace

In the third case the puncture of the dura mater was discovered after the injection of 2.8 ml bupivacaine-adrenaline The patient was a 30-year-old nullipara with a normal pregnancy A continuous lumbar epidural block was performed for the first stage of labour because of painful and incoordinated uterine contractions No abnormalities were recorded after the first dose of 2 ml but less than one minute after the second injection of 8 ml 0.25% bupivacaine-adrenaline the pain disappeared and the patient's systolic blood pressure fell to 75–80 mmHg The FHR (by stethoscope) which previously had been normal fell to about 80 beats/minute and the level of the anaesthesia reached Th IV The blood pressure returned to normal within 5 minutes with i.v. fluids and lateral positioning of the patient

Table III Indications for instrumental delivery and epidural block in labour

	No	Fetal asphyxia	Uterine inertia	Complications
Nulliparae	106	25	80	1
Multiparae	6	1	5	0
Total	112	26	85	1

Table IV Indications for caesarean section after epidural block in labour

	No	Fetal asphyxia	Threatened rupture of the uterus	Narrow pelvis	Uterine inertia
Nulliparae	16	9	2	4	1
Multiparae	1	1	0	0	0
Total	17	10	2	4	1

After a short period of moderate fetal tachycardia a new episode of accentuated FHR tachycardia was recorded and a caesarean section performed for impending fetal distress. The baby scored Apgar 9 at one minute. A couple of days after the operation the patient complained of severe post spinal headache.

In 3 cases the anaesthetic solution was probably injected directly into an epidural vein. The patients suffered with hypertension, tachycardia, pallor and unconsciousness for a couple of minutes after the injection of 2+8 ml 0.25% bupivacaine/adrenaline but without symptoms after about 5 minutes. After the catheter had been repositioned adequate analgesia was obtained.

In 3 cases the systolic blood pressure below 100 mmHg was recorded in 30 patients mostly after the first epidural injection but occasionally after reinjection. Patients were treated in a lateral position and with rapid intravenous infusion of fluids. In two patients 25 mg of ephedrine was given intravenously in addition to this treatment. The blood pressure returned to normal quickly in all cases.

Caesarean section and delivery

Instrumental delivery by vacuum extraction was used in 37.8% of the cases. Instrumental delivery

was less frequent in nulliparae than multiparae (Table II). The indications for instrumental delivery are given in Table III. During the same period the frequency of instrumental deliveries at this department was 8.3%. Patients with epidural block and caesarean section excluded.

Caesarean section was performed in 17 cases (Table II) which represents 5.8%. The indications are given in Table IV.

Nine per cent of the babies were born in occipito-posterior position (Table V) and corresponding figures in patients without epidural block was 3.4% during the same time period.

Oxytocin stimulation was used in 122 patients before as well as after the epidural block and in 55 patients after the blockade.

The time interval between the induction of analgesia and delivery was four hours seven minutes (range 24 hours 44 minutes—20 minutes).

Clinical condition of the newborn

The Apgar scores of the newborn at birth and after 5 and 10 minutes are given in Table VI. There were two cases of stillbirth, one case of hydrops fetalis due to Rh incompatibility and one case of hydrocephalus which was perforated during labour. Two further babies had multiple malformations.

Table V Frequency of occipito posterior and occipito anterior position at delivery after epidural analgesia for labour

	Normal delivery	Occipito posterior		Occipito anterior	
		No.	%	No.	%
Nulliparae	736	71	8.9	215	91.1
Multiparae	43	4	9.3	39	90.7
Total	279	75	9.0	254	91.0

Table VI Apgar score of the newborn after delivery with epidural block

Apgar score	At birth		5 minutes		10 minutes	
	No.	%	No.	%	No.	%
8-10	771	91.5	289	97.6	297	98.6
5-7	20	6.8	5	1.7	7	0.7
0-4	5	1.7	7	0.7	7	0.7
Total	96	-	296	-	296	-

Stillbirths (congenital malformations)

which required emergency surgery. Both died within one week after delivery. All the other children were discharged in good health from the hospital.

DISCUSSION

Lumbar epidural block provides an effective pain relief during the first stage of labour and is a well documented technique (4, 5, 6). Bupivacaine, a long acting agent, was used as the local analgesic in this study. From a pharmacological point of view bupivacaine seems to have certain advantages in obstetrics (2, 3, 11, 12) and the relatively long time interval between each epidural injection is in our opinion an advantage when pain relief is needed for a long period.

Bupivacaine with adrenaline was used in this study mainly because adrenaline free solutions were not available when this investigation was started. The addition of adrenaline does not seem to prolong the analgesic action of the drug but a certain reduction of the blood concentration can be seen and in cases requiring a prolonged analgesia the addition of adrenaline could be beneficial (13).

In this study the success rate was over 90% in the first stage of labour which is in agreement with other studies (6, 9). Pain relief was less effective during the second stage of labour. This is probably due to the technique used with small volumes of bupivacaine to minimize pelvic floor relaxation and preserve the bearing down reflex. A higher success rate during the second stage of labour can probably be achieved with larger volumes and more concentrated drug solutions. This would however probably increase the frequency of instrumental deliveries.

The suture of an episiotomy or the exploration of the uterine cavity post partum can easily be done after a top up injection of bupivacaine.

Dural puncture and inadvertent intravenous injection of the local anaesthetic agent are known complications of this technique (8). When these types of complications are properly diagnosed and treated no permanent damage to mother or child should occur but constant supervision of the patient is essential. In this study one case of unrecognized high spinal anaesthesia occurred which is a potentially dangerous complication when not properly treated. The test dose of 2 ml used is probably too small and is no guarantee against an

intravenous subdural injection of larger volumes of the local anaesthetic agent.

The incidence of hypotension is similar to that found in other investigations (5, 7). Lateral positioning of the patient and intravenous fluids is a preventive treatment in the majority of the cases. Hypotension is no major problem with this technique. Diagnosis and treatment of this condition should however be adequate to avoid compromise.

After an epidural block the mother will be conscious of uterine hyper- or hypotonia. Control of labour should therefore be very tight and cardiotocography is probably the best supervising method for mother and child. In this series cases of threatened rupture of the uterus occurred which definitely stresses the importance of a tight control of the patients. Borderline pelvic dimensions as evaluated by X-ray pelvimetry is in our opinion a relative contraindication to epidural block. Cervical stimulation of labour is probably more effective after epidural block which also emphasises the need for adequate supervision.

An increased frequency of instrumental deliveries will occur after epidural block. This phenomenon is probably mainly due to the relaxation of the perineum with a decreased bearing down reflex. It is therefore important that mothers and midwives are properly instructed. Because the decreased bearing down reflex seen after epidural block, breech position and multiple pregnancies represent in our opinion a relative contraindication to this type of analgesia.

The reason for the increased frequency of occipito-posterior position after epidural block is difficult to evaluate. Rotation of the fetal head may be disturbed by the pelvic floor relaxation seen after epidural block (4) but on the other hand the occipito-posterior position may in itself present a rather painful labour and these cases could be represented in this series.

Apgar scores were within normal limits after epidural block and the frequency of depressed babies was similar to that found in the control group and during the same period after general obstetrical analgesia (1). In view of the apparent lack of neonatal depression following epidural block with bupivacaine is in our opinion a safe method when effective pain relief is required in labour.

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FETAL MOVEMENTS DIAGNOSED BY ULTRASOUND IN EARLY PREGNANCY

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Abstract The purpose of this work was to ascertain whether ultrasonic techniques based on fetal movements are suitable for examinations in early pregnancy. The series consisted of 174 patients who came to be examined because of bleeding on the 6th–20th gestational week. The rapid B scan method detected the fetal movements from the 8th week onwards and the results were 100% reliable from the 10th week onwards. The combined A–B scan method detected the fetal movements from the 17th week. Techniques based on the detection of fetal heart function were used as control methods. According to our results, methods based on the indication of fetal movements constitute a practical alternative for elucidating the problems of early pregnancy.

During the last few years ultrasonic examination has established its position in the diagnosis of early pregnancy and its complications. By means of the B scan method intrauterine pregnancy can be detected from the 6th gestational week onwards. This method can also be used to measure the growth of the gestation sac and the uterus in early pregnancy. The use of the B scan technique, which is the most advanced ultrasonic method, is restricted in early pregnancy by the fact that it does not show directly whether the fetus is alive or not. Attempts have therefore been made during recent years to develop new ultrasonic methods or to combine existing ones in such a way as to show fetal heart function (5, 6, 7, 11). These methods are, however, rather relatively elaborate ultrasonic techniques or combinations of several methods and hence require considerable experience of the examiner. In addition to displaying fetal heart function, fetal movements can also be verified in early pregnancy by de-

tecting fetal movements. This can be done by two ultrasonic methods. One of these is the combined A–B scan, in which an ultrasonic beam is directed towards the fetus on the basis of the information shown by the oscilloscope of the B scan apparatus and the movements of the fetal echoes on the A-oscilloscope then show the fetal movements. The other technique is based on the rapid B scan method¹ whereby fetal movements are directly observable on the oscilloscope of the apparatus. In order to find out whether the mere diagnosis of fetal movements in early pregnancy can lead to a sufficiently early verification of fetal life, we compared the ultrasonic techniques showing fetal movements with those used to display fetal heart function.

MATERIAL AND METHODS

The patient series consisted of 174 patients admitted into the Department of Obstetrics and Gynecology, University of Oulu, on the 6th–20th gestational week because of bleeding.

In each case a rapid B scan was obtained first using the Vidison® (Siemens) apparatus (Fig. 1). In this apparatus a rotating probe transmitting and receiving ultrasound (2 MHz) gives two-dimensional cross section images of the object on the oscilloscope at such high frequency (16/sec) that spontaneous fetal movements can be demonstrated. The liquid filled probe of the apparatus can be placed in different positions above the object. Oil was used as a contact medium between the probe and the skin and the full bladder technique was employed to localize the uterus. The first objective was to detect fetal echoes in the uterus. After this had been done their possible movements were followed for 5 minutes when necessary (Figs. 2 and 3). In the other method of verifying fetal movements the combined A–B scan method (Kretztechnik's 4100 MGS apparatus) was used (Fig. 4). A 2 MHz ultrasound was directed towards the fetus on the basis of the information shown on the B scan oscilloscope. The fetal echoes were

Terms "split image technique" and "rapid picture" are also used.



Fig. 1 Equipment (Vidoson®) based on the rapid B scan method. The liquid filled probe on the patient's abdomen.

then seen as vertical echo peaks on the A-oscilloscope of the same apparatus (Fig. 5). The rapid horizontal shifts of these echoes were taken as signs of fetal movements.

Three control methods showing fetal heart function were used. The first of these was the combined A-B scan with Kretztecknik's 4100 MGS apparatus and a 2 MHz probe, whereby after the initial information obtained from the B scan the detection of the fetal heart function



Fig. 2 Longitudinal rapid B scan of the uterus in the 9th week. The examination is based on the possible movements of fetal echoes (F) on the oscilloscope. Bl = bladder, GS = gestation sac.



Fig. 3 Longitudinal rapid B-scan in the 14th week. Echoes of fetal head (H) can be seen in the Bl = bladder.

was possible as a typical rotating movement on the A-oscilloscope (Fig. 6). The other methods used were fetal Doppler examination by means of Pickers' apparatus and a 5 MHz probe (5) and a combined A-B scan and Doppler examination with Kretztecknik's 4100 apparatus supplemented by Pickers' EUD-1 carried out transabdominally by a 5 MHz probe.

If one of the methods employed showed the fetus alive on the first examination, no further was made. In the other cases, examinations were intervals of 1–2 weeks, until fetal life was pregnancy terminated spontaneously. From the onwards, however, no repeated examinations. Two examinations were made in altogether 13 cases, three examinations in 8 cases. Molar pregnancies were excluded from the series. The 10 of the patients were further followed by quantitative daily urine HCG assays and of the subsequent course of the pregnancy.

RESULTS

In the series investigated (174 patients), 104 ultrasonic examinations and subsequent follow-up showed continuation of the pregnancy in 101 (57.2%). In the remaining 53 cases (44.8%), pregnancy terminated in a spontaneous abortion.



Fig 5 Combined A-B ultrasound equipment (Kretztech 100 MGS) Longitudinal examination

these cases the diagnosis was missed abortion. Uterine evacuation revealed a dead fetus on the 19th week of pregnancy. In 3 cases out of 7 signs of fetal life had previously been demonstrated by ultrasound. The size of the fetus suggested however that death had taken place at the scan. In the other cases which terminated in spontaneous abortion no signs of fetal life had been detected by ultrasound. Most of them turned out to be cases of blighted ova.

The results obtained with the present ultrasonic methods are shown in Table I. The rapid B scan detected the first fetal movements in the 8th gesta-

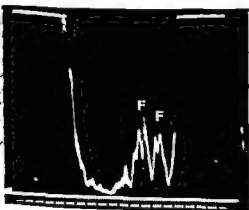


Fig 7 Fetal echoes (F) on the A-oscilloscope. The rapid horizontal movements of the echoes indicates the fetal

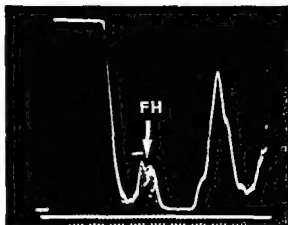


Fig 6 Fetal heart action (FH) on the A-oscilloscope. Echoes of fetal heart seen as a typical rotating movement

tional week in 3 cases. From the 10th week onwards the results were 100% correctly positive or correctly negative. No false positive findings were obtained. This method showed the fetal movements to be slight on the 8th-9th gestational week, and their detection often required a long time. It was also relatively difficult to see the fetal echoes in the gestation sac at this time. From the 10th week onwards the intensity of fetal echoes, the frequency of movements, and the duration of each series of movements clearly increased until the end of the period of investigation. Though the fetal movements were not analyzed in any more detail, two types of movement could be distinguished. The first type included violent movements of the whole fetus, which changed entirely its position in the amniotic cavity. The other type consisted of smaller movements, probably of the limbs, after which the fetus retained its previous position in the amniotic cavity. The period of immobility between movements was shorter in the first type. In the cases where no movements in the uterus could be detected, the oscilloscope showed whether the case was one of intrauterine pregnancy, whether the size of the gestation sac was equivalent to the duration of pregnancy or smaller, and whether fetal echoes were detectable in the gestation sac or whether the sac was empty.

Fetal movements were detected later by the combined A-B scan method than by the rapid B scan. The first movements of fetal echoes were observable in the 10th gestational week, and full reliability was only achieved from the 12th week.

Table I The percentages of correctly positive and correctly negative findings from the total α -fetoprotein examinations (N) made by ultrasonic methods to show fetal life in the 6th-20th gestational week, patients with symptoms of threatened abortion (153 examinations)

Ultrasonic method	Gestational weeks							
	6th N=5	7th 9	8th 16	9th 18	10th 29	11th 32	12th 71	13th-20th 73
Rapid B scan	0	0	19	50	100	100	100	100
Combined A-B scan	0	0	0	0	24	72	100	100
Methods based on the detection of fetal heart function	0	12	75	94	100	100	100	100
Used to detection of fetal movements								

onwards (Table I). In this method too the horizontal movements appeared to become the stronger and the more easily discernible the longer was the duration of pregnancy. No false positive findings were obtained. The B scan oscilloscope further permitted the usual diagnostic observations on the size of the gestation sac and the uterus and on the fetal echoes.

The control methods based on the detection of fetal heart function by ultrasound yielded the first positive findings in the 7th gestational week in the same series of patients. The findings were almost completely reliable from the 9th week: a false negative finding was only obtained in one case out of 18. The findings were 100% correctly positive or correctly negative from the 10th gestational week onwards.

DISCUSSION

The present findings showed that ultrasonic methods based on fetal movements indicate fetal life from the 10th gestational week onwards. Although the rapid B scan method detected fetal life in the 8th and 9th gestational weeks less often than did the ultrasonic methods based on fetal heart function, both techniques yielded 100% diagnostic reliability in the same week (the 10th gestational week). The findings obtained by rapid B scan are also very clear and illustrative. The combined A-B scan method revealed fetal movements about two weeks later than rapid B scan. One explanation for this might be that ultrasound directed by the former method only to the fetal body may not reveal movements which involve the limbs alone. The movements of fetal echoes in positive cases, however, are clearly interpretable in the A-B scan

method and the examiner finds it easy to detect movements after a few positive findings.

The fetal movements diagnosed in the present investigation proved to be similar to those described previously by Reinold with the rapid B scan method (8, 9, 10). Our own experience is limited exclusively to abnormal pregnancies and we therefore know little of the fetal movements in early pregnancy. Reinold (9) showed however restricted spontaneous fetal motility in the results in the birth of a healthy infant in only 4 of the cases, while the prognosis for infants with fetal motility is good in 94%. He (10) demonstrated that absence of fetal response to intrauterine manipulation signifies a poor prognosis for the fetus, even if signs of life were visible at the time of the examination. According to Reinold the observation period must last for at least 5 minutes. Haller et al. (3) who employed the ultrasonic technique were able to show the frequency and duration of fetal movements significantly reduced in pregnancies subsequently terminating in an abortion than in cases with a favourable prognosis. It is hence possible that the rapid B scan method may give prognostic information for the subsequent course of the pregnancy.

The diagnosis of fetal movements by the method described above is a new advance in the early pregnancy. It has been shown previously that fetal movements begin at a very early stage. Lepiane (1) demonstrated fetal movements at an age of 8 weeks in a gestation sac removed from a tubal pregnancy. Muscular contractions have been observed in fetuses 6 weeks old. Szendi (12) demonstrated fetal movements in the gestation sac during the first four months of pregnancy by contrast medium and X rays. The ultrasonic

employed in the present work did not reveal fetal movements until the 8th gestational week because fetal echoes could be seen prior to this time. The acoustic properties of the fetal tissues at an early age differ so little from the surrounding amniotic fluid that the detection of the fetal echoes requires particularly sensitive equipment (11).

The ultrasonic research into early pregnancy carried out during the last few years has focussed increasingly on the demonstration of signs of fetal life.

The main reason for this has been the fact that when the B scan method alone is used, diagnostic reliability after a single examination is only 78–87%.

4) Absence of fetal heart function from the 10th gestational week onwards on the other hand is a certain sign of a poor prognosis for the pregnancy (5–7). Robinson (11) achieved complete diagnostic reliability from the end of the 7th gestational week onwards by using a combined B–A–TM method in the demonstration of fetal heart function. On the basis of the present results it appears that a diagnosis of fetal movements leads to 100% diagnostic reliability in the verification of fetal life sufficiently early i.e. from the 10th gestational week onwards. The present work also confirmed a previous observation (5) showing that verifiable signs of fetal life signify a favourable prognosis for pregnancy despite symptoms of imminent abortion in over 90% of the cases. Since more often the findings are perhaps easier to interpret when the ultrasonic examinations based on fetal movements than in the other ultrasonic examinations of early pregnancy these methods particularly the rapid B scan method can be considered as suitable alternatives in the choice of ultrasonic techniques for studying early pregnancy. As the findings are unambiguous and easy to interpret the method can also be recommended for examiners who have little experience with ultrasound.

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CHANGES IN THE CERVICAL BLOOD FLOW DURING LABOUR

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Abstract The cervical blood flow changes during labour in pregnant women (15 cases) have been studied by local injection of ^{133}Xe into the cervix. The blood flow changes were compared with the external uterine pressure which was measured with a tocograph. During contractions the activity decreased temporarily but increased again between contractions. The overall decrease was greater in the control group (4 cases) than during spontaneous contractions. The conclusion is that relative cervical blood flow is rapidly changed during labour.

group with the maximal peak the delivery and babies were normal and in the other groups there were pathological findings.

We have studied the blood flow in the cervix during labour correlated with regular contractions. The results are expressed qualitatively.

METHODS AND MATERIALS

The tracer used was ^{133}Xe . The dose was 100 μCi in 0.1 ml saline solution. The tracer was injected slowly (15 sec injection period) into the cervix between contractions and the long needle was kept in the muscle for 10-15 seconds after injection. The collimator with NaI-crystal was placed over the symphysis.

One channel pulse height analyzer recorded the result on a chart.

At the same time the uterine pressure was measured with a Hewlett Packard external tocograph.

The measuring time was about 15 minutes.

The patients included 10 normal pregnant women, 5 toxemic women during regular spontaneous contractions and 4 normal pregnant women without contractions.

RESULTS

The level of radioactive tracer ^{133}Xe decreased during regular contractions. Between contractions the radioactivity accumulated again. However the changes happened a little after the peak of the contraction and after maximal relaxation (Fig. 1). During a contraction the cervix probably became a blood-free area and afterwards blood and ^{133}Xe flowed back.

The curves for the 2 women (X and Y) without contractions are illustrated in Fig. 2. Comparing the curve during contractions with the one recorded without any contractions we found that the decrease of radioactivity in the last curve was deeper and more rapid.

blood flow through the uterus and the placenta have been studied with different methods. The individual variations in the results are wide and depend on the duration of the pregnancy and on its complications. Studies with isotopes have provided much useful information. Munck et al. (8) first used ^{133}Xe for local injection to assess the circulation in uterine blood (13 ml/100 g/min). With the same technique Lehm et al. (2) observed a progressive decrease in clearance as pregnancy progressed. Falk et al. (1) studied the effect of the distance of the injection point from the placenta. Pontonnier et al. (3) observed near the placenta the initial rapid phase (1.0 \pm 0.8 ml/100 g/min) and the slow phase (0.3 ml/100 g/min) which was nearly equal to blood flow in the non-placental side of the myometrium. Jansson (4) did not observe any difference in the clearance rate between different parts of the non-pregnant uterine body and it was equal in pathological and normal pregnancies. Laakso et al. (5) observed with the scintillation camera that perfusion reached a maximum at the 34th week and fell to a low level by the 40th week but no difference was found between normal and pathological pregnancies. Also Janisch and Leodolter (3) distinguished three groups of patients on the basis of the type of flow curve. In the first

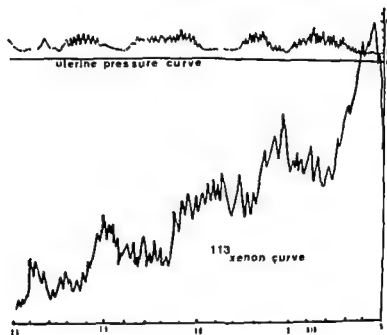


Fig. 1 The pregnant woman during contractions

DISCUSSION

Morris et al (7) and later Payling Wright et al (11) observed by using radioactive sodium that the uterine blood flow decreased both during exercise and labour.

Scheffs et al (10) analysed by computer the placental flow curve during labour finding that the blood flow starts to increase during the relaxation phase and continues up to an intrauterine pressure of about 30 mmHg in the next contraction. This increase is related to venous compression in the presence of a normal arterial flow. Shortly after the peak of a uterine contraction the blood flow again starts to increase. Although these measurements were made in the uterine body the same results can be obtained from the cervix. The blood flow in the cervix is even higher than in the uterine body whereas the blood pool in the placental side of the uterus is about twice that in the non placental side. Radioactivity returns to the cervix between contractions from this pool, probably by back flow. Some xenon labelled blood is also lost into the general circulation during a contraction. This distribution from the cervix, however, is slower during contractions than in a patient without contraction according to our figures.

The transport of the tracer from the injection point can vary because it is impossible to know the relationship between the injection site and the ves-

sels near which the transport is most rapid. Because this affects the quantitative measurement of the blood flow we have not calculated it although it is possible to do so by using Lassen's method for muscle blood flow.

These physiological findings are in agreement with previous clinical impressions. However,



Fig. 2 Two pregnant women (1) and (2) during contractions at term

it is possible to follow qualitatively the relationship between external uterine pressure and cervical blood flow

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UNCHANGED TOTAL BODY CALCIUM IN NORMAL HUMAN PREGNANCY

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Abstract The total body calcium—estimated from bone mineral content in the distal part of the forearm measured by photon absorptiometry—was determined during pregnancy in 13 women. The results indicate that total body calcium is constant during pregnancy.

The skeleton of newborn human infants contains about 25 g calcium and 80% of this amount is laid up in the last trimester of pregnancy (8). Physiologically pregnancy is considered a state in which calcium is lost from the maternal skeleton (1). Light & Reifstein (1) postulated that pregnancy and lactation were factors that cause osteoporosis in women. A high incidence of osteoporosis in multiparous women compared with parous has been reported by some investigators (3). Other authors were unable to find this difference (6, 9).

Linkson & West (2) have demonstrated by use of photon absorptiometry that lactating women lose about 1.2% of the total body calcium per 100 days. Photon absorptiometry of the distal part of the forearm is a reasonably good estimate of total body calcium, especially in well defined groups of patients (3, 4).

We report here a prospective study in pregnant women which aims at an evaluation of a possible change in total body calcium during pregnancy.

MATERIAL AND METHODS

Eleven women attending the out-patient clinic at the Department of Obstetrics and Gynecology at Glostrup Hospital took part in the study. They had all been pregnant 3-4 months at the beginning of the investigation and the women had symptoms of renal or digestive disease. Clinical data are given in Table I.

The bone mineral content (BMC) was determined by direct photon absorptiometry on both forearms (Fig. 1). The forearm is fixed in a Plexiglass container filled with distilled water. The source of radiation ($^{25}\text{mCi } ^{251}\text{I}$) and the detector are fixed in a holder at each side of the bones to be examined. A mechanical scanner displaces the detector and the source perpendicular to the longitudinal axis of the bones. The transmission of photons through the bones depends on the bone mineral content. Because the linear attenuation coefficients of Plexiglass, distilled water and soft tissue are almost identical the measurement is independent of the amount of soft tissue. In our modified version the BMC is expressed in arbitrary units as the mean value of six scans from each forearm. BMC of the forearm is well correlated to the body's total calcium content especially in normal subjects (4, 5).

Initially (at time t_0) BMC was measured. Thereafter the measurements were repeated at time t_1 (at 5-6 months pregnancy), at time t_2 (at 7-8 months pregnancy) and at time t_3 (at 9-10 months pregnancy). After the last measurement the women were questioned about their extra daily calcium supply during pregnancy (tablets and/or milk). Values for this supply are given in Table I.

As BMC is a function of sex and age (4) the BMC values of the pregnant women are expressed as per cent of the corresponding normal mean (Table II). To evaluate a possible change during pregnancy the initial value in each patient was termed 100 per cent and the following BMC values were recalculated to per cent of the initial value (Fig. 2).

Table I Height, weight, age, previous pregnancies and daily calcium supply in 13 pregnant women

	Mean	Range
Height (cm)	164.2	150-174
Weight at time t_0 (kg)	67.4	52-86
Age (years)	26.3	21-35
Previous pregnancies	1.2	0-2
Daily calcium supply (mg)	650	300-1200

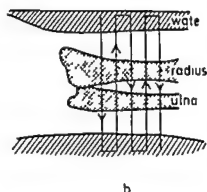
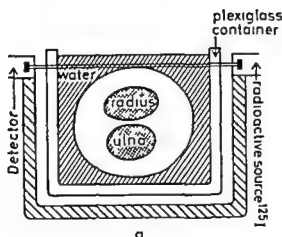


Fig 1 Method of measurement of bone mineral material content by direct photon absorptiometry on forearm (A) Section through system showing Plexiglass container and

U shaped holder with radioactive source and (B) System seen from volar surface showing movements of source and detector

RESULTS

In Table II the BMC expressed in per cent of the corresponding normal mean are given as a function of time. None of the mean values (at time t_1 , t_2 and t_3) are significantly different from the normal

mean (100%). Fig 2 shows the BMC values in per cent of the initial value during the study. Expressed in this way the BMC did not change.

DISCUSSION

The present results indicate that women given an extra supply of calcium do not lose from the skeleton during pregnancy. In other words, that women do not develop osteoporosis during pregnancy. A significant fall in BMC of lactating women can be demonstrated only if the lactation period exceeds 3 months (2). As this is rather the exception rather than the rule in Denmark, it is improbable that pregnancy and lactation are a high risk as regards the development of osteoporosis.

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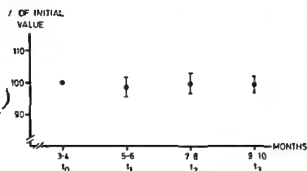


Fig 2 Bone mineral content (BMC) in 13 pregnant women as a function of time (t_1 , t_2 and t_3 corresponds to length of pregnancy of 3-4, 5-6, 7-8 and 9-10 months respectively). BMC values given as per cent of the initial value. Each point represents mean \pm 1 standard error of mean.

Table II Bone mineral content (BMC) in per cent of corresponding normal mean (x) as a function of time in 13 pregnant women. Values are given as mean \pm 1 S.E.M.

	3-4 months (t_0)		5-6 months (t_1)		7-8 months (t_2)		9-10 months (t_3)	
	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
BMC	100%	10.1	99.7	4.3	100.7	3.5	100.9	4.1
x								

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PHOSPHOLIPIDS AND CREATININE IN AMNIOTIC FLUID IN RELATION TO GESTATIONAL AGE

II Complicated Pregnancy

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act 176 samples of amniotic fluid were obtained by minimal amniocentesis from 69 women with complicated pregnancies (toxemia diabetes mellitus or rhesus immunization) in the 27th-43rd week of pregnancy. The concentration of creatinine (172 cases) the ratio lecithin/sphingomyelin (L/S ratio 155 cases) and phospholipid phosphorus (155 cases) were determined and related to gestational age. The results were compared with normal pregnancy. The mean level of creatinine in toxemia was significantly higher from the 37th week till term (0.05) and the L/S ratio was significantly higher (0.05) in the 34th-35th week compared with normal pregnancy. Creatinine concentration was significantly elevated in diabetic pregnancy in the 36th-37th week (0.05). Creatinine concentration tended to be low in immunization. A creatinine concentration ≥ 1.8 and a L/S ratio ≥ 2.5 always corresponded to a gestational age ≥ 35 th week. To get increased precision in dating gestational age in complicated as well as in normal pregnancy the determination of both creatinine concentration and L/S ratio in amniotic fluid is recommended.

accurate estimation of gestational age and fetal maturity is essential prior to elective induction or elective cesarean section and for the evaluation of high risk pregnancies such as toxemia Rh immunization diabetes suspected postmaturity placental insufficiency. Current methods for estimation of gestational age and fetal maturity may fail with serious consequences especially in high risk pregnancy. In a previous study we found that the determination of the lecithin/sphingomyelin ratio (L/S ratio) and creatinine concentration in amniotic fluid samples obtained by amniocentesis could be used for estimation of gestational age with good precision.

In the present investigation we have determined the lecithin/sphingomyelin ratio (L/S ratio) the concentration of creatinine and phospholipid phosphorus in amniotic fluid in different groups of complicated pregnancy and have related the results to the gestational age. As was the case in normal pregnancy the combined determination of creatinine concentration and L/S ratio made possible the determination of gestational age with good precision in complicated pregnancy.

MATERIAL

The group includes all patients with complicated pregnancy according to Table I who were treated and delivered at the University Hospital of Umeå during the period February 1972 until June 1973. Gestational age was estimated according to criteria given earlier (10). Women with uncertain gestational age were omitted. Amniotic fluid was obtained by abdominal amniocentesis. When all

Table I Distribution of material

	Patients	Samples
Toxemia		
Mild/moderate	1	2
Severe	8	11
Chronic hypertensive vascular disease in pregnancy	9	16
Diabetes mellitus White (A, B and C groups)	7	23
Diabetes mellitus White (D, E and F groups)	4	12
Rh immunization	29	9
Total	69	176

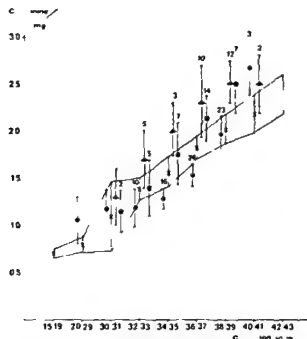


Fig. 1 Creatinine concentration (mg%) in amniotic fluid during gestation in complicated pregnancy. Each sign represents the means of the number of determinations given in the figure. The vertical bars indicate the 95% confidence interval for each mean. The 95% confidence interval for normal pregnancy is indicated by drawn lines. x Normal pregnancy, Δ toxemia, \bullet iso-immunization, \blacksquare diabetes.

specimens contaminated with blood or meconium had been discarded there remained 176 specimens of amniotic fluid from 69 patients to be examined. All specimens were coded and tested blindly. The groups studied and the number of patients in each group are shown in Table 1.

Diagnosis and classification of toxemia of pregnancy was done according to a recommendation of the Committee on Maternal Welfare issued in 1961. The diagnosis was based on the presence of at least two of the following symptoms and signs: (A) a rise in blood pressure of >90 mmHg in several occasions; (B) persistent moderate to marked edema; (C) proteinuria $\geq 1+$ protein (Albustix) in midstream specimen.

The patient was specified of having severe toxemia if one or more of the following criteria were fulfilled: 1) Resting diastolic blood pressure >100 mmHg; 2) Treatment with hydralazine ≥ 200 mg daily; 3) Proteinuria $>3+$ protein. All patients were treated with chlorothiazide and all but 6 patients were also treated with hydralazine.

The White classification was used for the severity of diabetes mellitus (78).

All mothers with Rhesus isoimmunization had antibodies demonstrated by the indirect Coombs test. The live born infants were Rhesus positive and had a negative direct Coombs test.

Amniotic fluid samples were obtained in the second half of pregnancy. In most cases 2-4 samples were obtained at intervals of 1-3 weeks.

The values for L/S ratio and creatinine concentration in normal pregnancy were derived from our previous study (10).

METHODS

Amniotic fluid was obtained by abdominal paracentesis, centrifuged for 10 minutes and then filtered on a filter paper (10). Creatinine was analyzed colorimetrically according to Jaffe's method (3) and the supernatant was frozen and stored at -20°C until analyzed. Phospholipid content and L/S ratio (10) for creatinine determination failed for technical reasons in 21 cases; the determination of L/S ratio was also failed in 21 cases because of an insufficient amount of liquor.

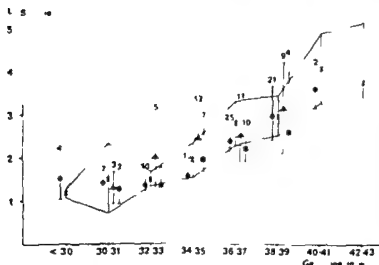


Fig. 2 Lecithin Sphingomyelin (L/S) ratio in amniotic fluid during gestation in complicated pregnancy. Each sign represents the means of the number of determinations given in the figure. The vertical bars indicate the 95% confidence interval for each mean. The 95% confidence interval for normal pregnancy is indicated by drawn lines. x Normal pregnancy, Δ toxemia, \bullet iso-immunization, \blacksquare diabetes.

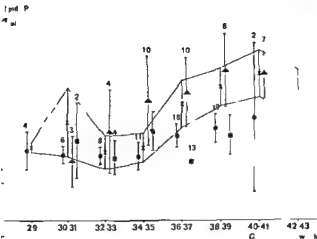


Fig 3 Concentration of phospholipid phosphorus in amniotic fluid during gestation. Each sign is mean \pm S.E. of the number of determinations given in the figure. The mean \pm S.E. for normal pregnancy is indicated by drawn lines. \times Normal pregnancy, Δ toxemia, \bullet iso-immunization, \blacksquare diabetes.

STATISTICAL METHODS

the assumption that the values in the normal series are normally distributed, prediction intervals for each time interval were calculated as

$$D \sqrt{\frac{n+1}{n}} t_{0.95} (n-1)$$

t ($n-1$) is the 97.5th percentile of a t -distribution with $n-1$ degrees of freedom. The probability that the value of a presumptive normal individual falls outside the interval is approximately 5%. For each of the pathological groups and for each time interval, the number of individuals with values outside the corresponding normal prediction interval was recorded. This number follows a binomial distribution with parameters n and 0.05, where n is the number of individuals in the pathological group examined during the specific

time interval. The probability of obtaining the observed result or a more extreme one was calculated. A small probability value indicates that the pathological group has a distribution of values which ought not to be regarded as normal.

RESULTS

Toxemia This group includes mild and severe toxemia and hypertensive disorders. No difference was found between the different subgroups of toxemia and chronic hypertension. The mean level of creatinine (Fig. 1) was higher in toxemia than in normal pregnancy of the same gestational age. The difference was statistically significant ($p < 0.05$) from the 32nd week of pregnancy till term. The

II Gestational age (n) and the combination of creatinine (Cr mg%) and L/S ratio in amniotic fluid in normal and complicated pregnancies

number of samples N = number of patients

	n	S	N	α of S			
				$\text{Cr} \geq 1.8$ and $\text{L/S} \geq 2.25$	Either $\text{Cr} \geq 1.8$ or $\text{L/S} \geq 2.25$		$\text{Cr} < 1.8$ and $\text{L/S} < 2.25$
					$\text{Cr} \geq 1.8$	$\text{L/S} \geq 2.25$	
normal	≥ 38	42	47	71	17	10	2
	37-36	5	75	40	1	24	74
	35-30	29	9	0	10	10	80
immunization	≥ 38	73	16	57	9	17	17
	37-36	25	73	12	12	40	36
	35-30	31	19	0	0	3	97
	≥ 38	15	14	87	13	0	0
	37-36	10	10	40	30	10	0
	35-30	19	13	5	59	14	77
	≥ 38	5	4	60	40	0	0
	37-36	13	10	46	23	73	8
	35-30	14	8	7	21	0	77

Table III Minimum values of L/S ratio creatinine/AF and gestational age under certain circumstances and complicated pregnancies

	Week of pregnancy ≥ 38		
	Minimum values of L/S ratio	Minimum value of creatinine/AF (mg %)	Minimum gestational age (weeks) at which L/S ≥ 2.5
Normal pregnancy	1.55	1.4	36
Iso Immunization	1.87	1.4	36
Toxemia	1.66	1.9	35
Diabetes	1.80	2.1	35

L/S ratio (Fig. 2) was significantly higher in toxemia during the 34th–35th week ($p < 0.05$) than in normal pregnancy but within the normal range at later times.

Rh isoimmunization There was a tendency to lower concentrations of creatinine from the 32nd week of pregnancy until term in this group compared with the normal group (Fig. 1). The difference was however not statistically significant. The L/S ratios did not deviate significantly from those in normal pregnancy (Fig. 2).

Diabetes No difference was found between subgroups of diabetes and they are thus presented as one group. The creatinine concentration (Fig. 1) in the 36th–37th week was significantly higher ($p < 0.05$) compared with the normal group. The L/S ratio fell within the normal range (Fig. 2).

The concentration of total phospholipid in the different groups (Fig. 3) showed mainly the same distribution as the normal group.

Table II shows the relation between the gestational age and the combination of certain borderline values for L/S ratio and creatinine concentration in different groups of high risk pregnancy. In toxemia only 22% of the samples before the 36th week had a creatinine value < 1.8 mg % and a L/S ratio < 2.25 . The immunization and diabetes groups showed a pattern similar to the normal group.

Table III shows the minimum values in pregnancy ≥ 38 th week. When the borderline values creatinine concentration ≥ 1.8 mg % and L/S ratio ≥ 2.25 were combined the length of gestation never underrated the 35th week neither in the normal nor in the pathological groups of pregnancy.

DISCUSSION

In the present report data on a relatively small number of patients are presented. Furthermore

several amniotic fluid samples have been obtained from most of the patients. Conclusions are therefore be drawn with care.

In toxemia creatinine concentration was significantly increased ($p < 0.05$) from the 34th week till term over those in the normal group. This is in accord with recent reports (11, 27) but in contrast to others (8, 9, 11, 17). The reason for the lack of agreement between reports is not clear.

However differences in classification of patients in terms of severity, duration of the disease, of treatment etc. may be of importance.

The reason for the higher creatinine concentration in amniotic fluid in toxemia is not clear. It has been suggested that it might be due to a decreased rate of transport of creatinine from maternal serum creatinine concentration into amniotic fluid (27). It has also been suggested that treatment with thiazide diuretics may be of importance (20). Variation in the amniotic fluid volume probably does not affect creatinine concentration (24). The possibility that maternal serum creatinine may pass through the placenta and affect fetal function or that placental insufficiency or increased fetal catabolism has also been suggested.

In isoimmunization the amniotic fluid creatinine concentration (Fig. 1) tended to be lower than in this group and the normal group. It is interesting to note that Hinkley et al. (10) found significantly lower amniotic fluid creatinine concentration in isoimmunization with < 1.8 mg % often accompanying fetal malnutrition.

The amniotic fluid creatinine concentration in diabetes was within the limits of the normal group with the exception of the 36th–37th week when there was a significant increase ($p < 0.05$).

number of patients in this group is small however it is concluded that the isolated increase in creatinine concentration should be interpreted with care. Slight increase in creatinine concentration in diabetes has been reported (75) and was interpreted as a sign of a rapid maturation of the fetus in diabetes. However this conclusion might not be valid for the fetus as a whole as the L/S ratio which is an estimate of fetal pulmonary maturity (2, 13, 14) was within the normal range in diabetes (Fig. 2).

L/S ratio deviated significantly from normal only in toxemia and at one time only the 35th week (Fig. 2). In isoimmunization and diabetes the L/S ratio was within the normal range in accord with Doran et al. (9).

The early increase in L/S ratio in toxemia compared with normal pregnancy is in accord with the findings of Gluck et al. (12, 13, 15). They have also detected early increases in L/S ratio in a number of pregnancies complicated by maternal diseases other than toxemia. Others however have not found any increase in L/S ratio (6, 7, 9) or amniotic fluid lecithin concentration (1, 2) in any complicated pregnancy. In diabetic pregnancies (classes A, B and C) the increase in L/S ratio was delayed (13) and did not occur until 37 weeks of gestation. This has been observed by others (23, 29) but was not observed in our series (Fig. 2). As discussed above discrepancies may be due among other things to different ways of classification of maternal disease. In the case of the L/S ratio divergent results might also be due to methodological differences (10).

It has been suggested (19) that the synthesis of surface active lecithin in the lung (which is the precursor of most of the lecithin in the amniotic fluid) is under the influence of the adrenal corticosteroids and at this is why an earlier pulmonary maturity can be seen in pregnancies with intrauterine fetal death. On the other hand in pregnancy with an anencephalic fetus where adrenal function should be deficient both low (1) and normal (4) L/S ratios have been reported.

A high amniotic fluid creatinine concentration in toxemia is of great clinical importance. Thus in pregnancies of premature toxemia pregnancies (<36th week) had creatinine concentrations ≥ 1.8 mg%. It is thus a possible risk that the pregnancy will be prematurely induced before the fetus has reached pulmonary maturity if estimation of gestational age is based only on creatinine concentration (Table II). To get a more accurate estimate

of the gestational age the L/S ratio should also be determined as the use of these two measurements in combination only gives a 5% false positive rate (Table II). The risk of inducing delivery prematurely diminishes considerably in this way.

The tendency to low creatinine values in isoimmunization (Fig. 1) although the values were not significantly lower than normal might still be of clinical importance. If the estimation of gestational age is based solely on creatinine concentration some cases might be placed in an incorrectly low group according to Liley. This might obviously have severe consequences for the fetus. The situation can be avoided if the L/S ratio is also determined. Therefore we feel that when gestational age is unknown not only creatinine but also L/S ratio should be determined.

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VAGINAL REPAIR IN THE RADICAL OPERATION FOR CERVICAL CARCINOMA

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Abstract An attempt was made to preserve postoperative sexual function and the recovery of bladder function by a modification of technique in 22 cases of radical operations for invasive carcinoma of the cervix. The procedures consisted of two parts. After removal of the uterus, the bladder peritoneal flap is sutured to the anterior vaginal wall leaving a 2 to 3 cm margin between line of suture and the edge of the peritoneum. Similar to the posterior peritoneal flap is sutured to posterior vaginal wall leaving a margin. Both edges of the peritoneum are then closed forming a pouch as an extension of vaginal canal. The length of the vagina was successfully elongated by this procedure, thus adding to better postoperative sexual function.

Radical hysterectomy with pelvic lymphadenectomy has been commonly performed for the treatment of first and second stage carcinoma of the cervix in Japan. The surgical procedures were based principally on the Okabayashi method and operative techniques were further developed by No. Hashimoto, Magara, Kobayashi et al. The problems of radical hysterectomy are the primary mortality, urinary tract complications such as infection, fistula formation, ureteric stricture, bladder atony and shortening of the vagina (2, 3). The primary mortality rate, however, has diminished significantly and the incidence of retrovaginal, vesicovaginal and rectovaginal fistulae also markedly decreased by the improvement of operative techniques (4, 5). Remaining problems are the prevention of bladder atony, ureteric stricture and shortening of the vagina. Postoperative sex life is frequently disturbed as a result of a short vagina. The present study was carried out with the intent of preserving the sex life after the operation by modifying vaginal repair techniques and accelerating the recovery of the

bladder function by fixing the bladder to the vaginal wall.

MATERIALS AND METHODS

The total number of patients subjected to this modified technique was 22, including 9 patients with stage I-B, 1 stage II-A, 7 stage II-B, 9 stage III-B and 1 stage IV. These patients were managed surgically after the Okabayashi method at the Tokyo Medical and Dental University Hospital during 1971-1972.

The characteristics of the Okabayashi method are as follows: 1) At first the pelvic lymph nodes are removed by block dissection along the external iliac vein, obturator nerve, internal iliac vein and the cardinal ligament and down the side of the pelvic wall. 2) The uterine ligaments, e.g. cardinal, rectovaginal and vesicovaginal ligaments are isolated and dissected separately. 3) The ureter should be stripped off preserving the adventitia and fine paraureteric tissues especially in the region between its crossing with the uterine artery and the bladder. 4) The vagina can be sufficiently detached and dissected, a retractor including the areas of cancer infiltration so that the line of dissection is at least 2 to 3 cm clear of carcinomatous involvement.

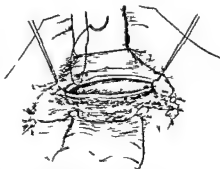


Fig. 1. Ligatures of No. 1 chromic catgut are placed on the anterior vaginal wall for hemostasis.

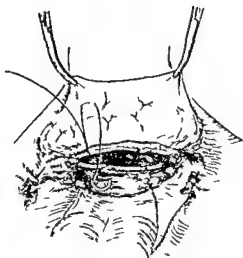


Fig 2 The bladder flap is attached to the anterior vaginal wall using the same suture of chromic catgut

In this study certain procedures are added to the original radical operation as follows: 1) The bladder peritoneum is detached at the uterine reflexion so that a sufficiently wide peritoneal cuff is left. Similarly the posterior peritoneum is dissected. 2) Vaginal repair is completed after the specimen has been removed. Three to four ligatures of No. 1 chromic catgut are placed on the anterior vaginal wall not including the vaginal epithelium for hemostasis (Fig. 1). 3) Then the same interrupted sutures are used to attach the bladder peritoneal flap to the anterior vaginal wall, the bites being taken 2 to 3 cm from the edge of the peritoneal cuff. When all the sutures are placed a margin of 2 to 3 cm of peritoneum is left free from sutures (Figs. 2 and 3). 4) In the same manner sutures are placed on the posterior peritoneum and the posterior vaginal wall (Fig. 4). 5) The edges of the anterior and posterior peritoneum are sutured together with interrupted 00 plain catgut to form a peritoneal pouch as an extension of the vaginal canal (Figs. 5 and 6). Two drains are inserted through the abdominal wall into the dead space on both sides deep in the pelvis.

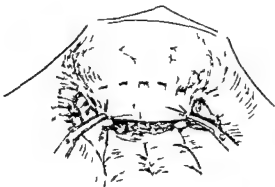


Fig 3 A margin of bladder peritoneal flap of 2 to 3 cm width is left free from sutures



Fig 4 Sutures are placed on the posterior vagina and the posterior peritoneum in the same manner

An indwelling catheter was used for 7 days after operation. The volume of residual urine after each void was measured thereafter. By the 10th to 14th postoperative day the peritoneum forming the vaginal pouch turned grey and adhesions formed readily. Serous and bloody discharge is observed. Digital separation and the application of antibiotics may be useful. The pouch gradually undergoes necrosis but in about 3 months the peritoneal pouch is completely replaced by new epithelium. The duration of admission is usually 1 to 2 months or more are required when extensive necrosis is performed.

Seventeen of 27 patients received postoperative pelvic irradiation to the pelvis. These 17 patients had metastases in the pelvic lymph nodes, parametrial or extensive infiltration of the cervix.

Patients were followed every 3 months by pelvic examinations. Renal function studies, cystoscopy, intravenous pyelography and other laboratory tests were done as required. Patients were advised to resume intercourse soon after leaving the hospital. The length of the vagina was measured about 17 to 19 years after operation and the woman's sex life was interviewed personally in detail. These results were compared

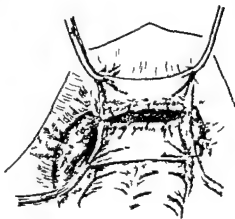
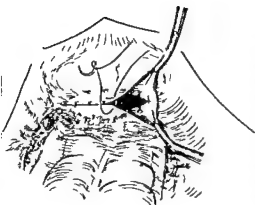


Fig 5 A margin of rectal peritoneal flap is kept free from sutures



6 The edges of the anterior and the posterior peritoneum are sutured together to form a peritoneal pouch as an extension of vaginal canal

of 36 subjects with radical operation for invasive cervical carcinoma performed during 1968 to 1970 with this modification

RESULTS

Measurement of the length was made along the anterior wall of the vagina. The mean of the length of the vagina in 22 patients was 6.6 cm ranging from 4.5 to 7.5 cm in contrast to 4.9 cm in 36 control subjects without this procedure. In the study group the vagina could be further lengthened another 1 to 2 cm by the application of pressure at the vault.

Spontaneous urination usually starts on the day after the indwelling catheter is removed. The mean time in the study group was 8.5 days after the operation whereas in the control group it was 12.6 days. It took 25.3 days on average before the residual urine was constantly less than 50 ml in the study group. The range was from 14 to 62 days. In the control group it was 27.6 days.

Of the 22 patients 8 (36%) reported their sex life as satisfactory and 9 (40%) noted some discomfort. No patients had serious complaints. In the control group 65% had a mild to severe sexual disturbance and 20% had no complaint in this regard. Ureterovaginal fistula was found in 1 out of 22 study cases and 1 in the control group. There was no incidence of vesicovaginal and rectovaginal fistula. One recurrence of carcinoma in the vaginal stump was found in the control group. Two of the study cases died with recurrence at the pelvic wall and metastases in the ascending lymphnodes respectively within 2 years of the operation.

DISCUSSION

The first principle to be observed in the surgical treatment of cervical carcinoma is a radical operation but its magnitude may lead to the occurrence of serious postoperative complications. Careful and complete removal of the pelvic lymphnodes is expected to raise the 5 year survival rate. The incidence of recurrence is higher in the presence of pelvic lymphnode metastases. Wide dissection of the vaginal wall and complete isolation of the bladder and ureter often results in fistula formation, urinary bladder dysfunction and shortening of the vagina. Wrapping the ureter with pelvic peritoneum or intraperitoneal replacement of the ureter has been employed to prevent ureteric fistula and postoperative hydronephrosis. These procedures can prevent displacement of the ureter toward the pelvic wall by scar formation and kinking and can also avoid its malnutrition when placed extraperitoneally.

The use of bougies, dilatation of the urethra, diathermy incision of the sphincter muscle at the urethro-vesical junction are reported to be helpful in relieving urinary symptoms caused by bladder atony. In this study the modification was made so that postoperative sexual disturbance due to shortened vagina and bladder dysfunction may be lessened. Improvement was found in vaginal length, vaginal flexibility and postoperative sexual function but the fixation of the bladder to the vagina did not improve bladder function. The use of peritoneum in the construction of a vagina was described by other authors (6). We also have one case of aplasia of the vagina treated by the same principle with a good result. The isolation and preservation of pelvic parasympathetic nerves from the cardinal ligament and paracervical tissue (7) and preservation of the vesical artery (8) were reported to facilitate recovery of bladder function especially in the early stages of cervical carcinoma. Adoption of these techniques is to be considered to obtain further improvement of bladder function.

Incidence of ureterovaginal fistula 1 out of 22 cases or 2 out of total 58 cases is not particularly low compared to other studies. Extensive invasion of the paracervical tissue and resulting difficulty in preserving paraureteric tissue may be the reason for the fistula formation.

Recurrence at the vaginal stump was found only in 1 out of 58 cases. This low incidence of local recurrence may be one of the characteristics of the

Okabayashi operation. However, a short vagina of about 4 to 5 cm in length is formed as a result if no modification of the original procedure is made. Generally, without modification, the vagina becomes fibrous and rigid after operation. Although lack of sex desire or avoidance of sexual activity after the operation was found in 5 of 22 cases (22%), improved sexual function realized by the new technique indicates that the major factor causing the sexual disturbance is the shortened vagina and lack of distension of the vaginal pouch.

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THE EFFECT OF A COPPER IUD AND VARIOUS 'INERT' IUDS ON THE HISTOLOGICAL PICTURE OF THE RABBIT ENDOMETRIUM DURING EARLY PSEUDOPREGNANCY AND PREGNANCY

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Abstract The influence of a copper IUD and two 'inert' IUDs on the histological picture of the rabbit endometrium during early pseudopregnancy and early pregnancy was studied. The copper IUD markedly inhibited endometrial maturation during the first five days of pseudopregnancy and pregnancy. The 'inert' IUDs had no such effect. The copper IUD prevented the normal increase of the rabbit uterus to 17 beta estradiol in oestrogen-treated animals. The observed effects of the copper IUD might seriously interfere with the normal process of implantation.

Several authors report that 'inert' IUDs in women have traumatic effects on the endometrium: local pressure atrophy, superficial endometrial necrosis, endometrial necrosis, stromal oedema, hyperplasia and dilatation of capillaries and haemorrhages (6, 7, 19, 20, 22, 24, 32, 35, 37, 47). Traumatic effects are also described in various experimental animals (2, 8, 9, 27, 28, 30). Most of these effects are localized in the proximity of the IUD. An increase in inflammatory cells in the endometrium and in the uterine lumen is usually always found in women (4, 6, 17, 18, 22, 24, 31, 37, 38, 39, 40, 48) and in experimental animals (10, 14, 21, 33, 36, 43) fitted with 'inert' IUDs. This inflammatory reaction was absent in the absence of infection (31). In rats and guinea pigs a copper IUD caused a more marked inflammatory response than did a plastic IUD (10). The relation between the number of inflammatory cells and the reduction of fertility has been investigated (33, 36).

The effect of IUDs on the normal maturation of the endometrium has been studied in several

species. Most of the reports on women wearing IUDs found no change in the normal development of the endometrium (17, 20, 24, 40). Evidence supporting a premature development was presented by Wynn (44, 45, 46) whereas others (4, 6, 37, 47) reported a delayed development. In rats the decidual reaction is inhibited by 'inert' IUDs (9, 12, 23, 26, 29) and by a copper IUD (41). Zipper in his original work (47) on the effect of a copper IUD on the rabbit endometrium reported an intense proliferation of the mucosal stroma. However, Abraham (1) found no morphological changes in the presence of a copper IUD during the first four days of pregnancy in the rabbit.

In two previous reports (15, 16) a copper IUD was found to inhibit the DNA synthesis in the endometrium during early pseudopregnancy and pregnancy in the rabbit. This suggests an improper maturation of the endometrium. The present study therefore investigated the effect of a copper IUD and various 'inert' IUDs on the morphology of the rabbit endometrium during the first five days of pseudopregnancy and pregnancy.

MATERIAL AND METHODS

Virgin rabbits of mixed breed weighing two to three kg were used. Three types of IUDs were studied: 1) Copper IUD—consisting of a 50 mm long polyethylene catheter 0.5 mm in outer diameter (Clay Adams, Parsippany, N.Y., USA) around which a copper wire 0.73 mm in diameter was coiled giving a surface area of 900 mm² (Cu IUD). 2) Platinum IUD—identical with the Cu IUD except for the change of metals (Pt IUD). 3) Plastic IUD—consisting of a polyethylene catheter 1.0 mm in outer diameter and 50 mm long (PI IUD).

Table 1 Presentation of various experimental groups with number of animals and main effects of types of IUDs

Group	Combinations of IUDs	Subgroups (hours)	No of animals	Main histological effects of IUDs
I	PI IUD ₁₀ SH (stimulated with HCG)	0	5	In horns fitted with the PI IUD ₁₀ as compared with operated horns (in all subgroups) erosion of mucosal necroses increased number of inflammatory
		48	4	
		170	3	
II	PI IUD ₁₀ PI IUD (stimulated with HCG)	0	4	In horns carrying the PI IUD as compared with containing horns increased number of eosinophilic were seen Otherwise the same changes as noted PI IUD ₁₀ but with more frequent mucosal necroses
		48	4	
		170	4	
III	PI IUD ₁₀ Cu IUD (stimulated with HCG)	0	3	In horns fitted with the Cu IUD as compared with containing horns at 0 hours swollen epithelial glands At 48-72 hours less pronounced proliferation of the surface epithelium less minor folds glands few mitotic figures At 170 hours reduced of minor folds with fewer interconnecting body glands In all subgroups increased inflammation and more frequent necroses
		48	5	
		72	5	
		170	5	
IV	PI IUD ₁₀ Cu IUD (mated animals)	0	7	In horns fitted with the Cu IUD the same changes described in Group III
		60	6	
		170	6	
V	PI IUD ₁₀ Cu IUD (castrated estrogen treated animals)	0	3	In horns fitted with the Cu IUD a poor response given with low cuboidal flattened epithelium glands mucosal necroses a marked inflammation and a reduced diameter of the uterine horn

The IUDs were sterilized by soaking in ethanol and rinsed in sterile physiological saline before use. The animals were anaesthetized with Nembutal and the abdominal cavity was exposed under sterile conditions via a lower midline incision. No corpora lutea were present at the operation. The IUDs were introduced into the uterine just above the utero-cervical junction, the lower of the IUDs being fixed to the uterine wall by a 4/0 silk suture at the puncture. Uterine horns designated sham operated (SH) were exposed to introduction and with drawal of the PI IUD₁₀, a 4/0 silk suture being placed in the antimesometrial wall.

The effect of the different IUDs was compared with the effect of the PI IUD₁₀. Thus one horn was always fitted with a PI IUD and the other with one of the other types of IUDs in all groups except in the group where one horn was sham-operated. The IUDs were always randomized between the horns. The rabbits were divided into five groups according to the combinations of IUDs (Table 1).

On the fifth day after operation, most of the animals were given 75 IU of HCG (HCG human chorionic gonadotropin Gonadex Leo AB Helsingborg Sweden) intravenously to induce pseudopregnancy or mated with fertile bucks. The animals were subsequently killed by cervical dislocation at various times up to 170 hours after injection or mating. Some animals—killed on the fifth day after operation corresponding to 0 hours—received no HCG injection. The mated animals were killed immediately after mating. In one group (Table 1 Group V) three animals castrated for at least two months and fitted with the PI IUD₁₀ and the Cu IUD were injected with 1

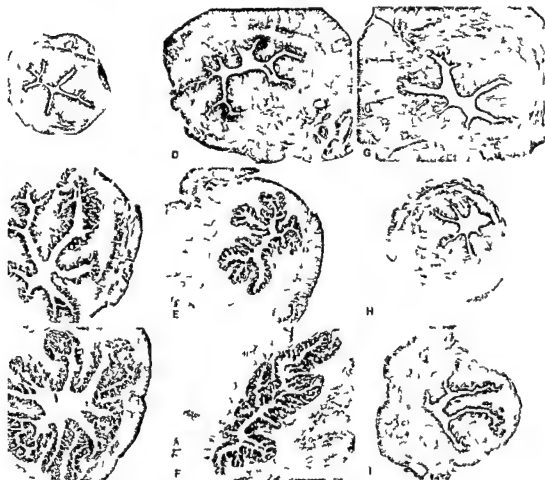
µg 17 beta oestradiol per kg bodyweight 170 hours beginning 48 hours after the insertion of the IUD.

The internal genitalia were taken out and the horns were trimmed. The presence of corpora in each ovary was registered. The uterine horns responded to the length of the IUD was a Cu IUD part. A part of the midportion of the IUD fixed in Bouin's solution. All specimens were embedded in paraffin and the sections were stained with haematoxylin and eosin.

RESULTS

In one group of animals one horn was sham-operated and the other was fitted with the PI IUD (Table 1 Group I). In sham-operated horns at 0 hours (unstimulated animals) Fig 1 major mucosal folds were lined by a simple columnar epithelium as were the few endometrial glands. Very few mitotic figures were present. The endometrial stroma had the appearance of loose connective tissue rich in small capillaries and prominent endothelial cells. Few inflammatory cells were seen.

At 48 hours (Fig 1 B) the surface epithelium was pseudostratified and the numerous and large glands were also partly lined by a pseudostratified



Maturation of the endometrium in sham-operated (Fig 1 A B and C at 0, 48 and 120 hours after stimulation respectively) in horns fitted with the D.I.U. (Fig 1 D E and F at 0, 60 and 120 hours post-stimulation) and in horns fitted with the Cup-IUD (Fig 1 G and H at 0, 60 and 120 hours post-stimulation) are no apparent differences between sham-operated

and PI IUD containing horns (cf Fig 1 A C to Fig 1 D F). In the presence of the Cu IUD there is an inhibition of the maturation of the endometrium as compared with PI IUD horns (cf Fig 1 G-I to Fig 1 D-F) the comparison is made between horns within the same animal ($\times 764$).

Minor folds appeared and in some areas between them were formed. Mitoses were present both in the surface and in the glandular lumina. In the minor mucosal folds the stroma appeared as thin septa. Numerous sinusoidal vessels were seen beneath the epithelium. Occasional interstitial oedema was seen in the stroma as swollen fibroblasts. Only very few leucocytes were present.

At 120 hours (Fig 1 C) the minor mucosal folds were now very pronounced as were the bridges between adjacent folds. The surface epithelium was pseudostratified whereas the glands were

mainly lined by a single layered columnar epithelium. Single mitotic figures were seen. Only a few specimens showed a columnar epithelium with protruding cytoplasm at the top of mucosal folds. A very thin stromal lining containing sinusoidal vessels was found between the epithelium of the mucosal folds and between the glands. No leucocytes were found.

In the contralateral horn of these rabbits fitted with the PI IUD, the above described histological picture was generally repeated (Fig 1 D E and F Fig 2 A B and C). However, at all times after HCG stimulation areas of local erosion sometimes



Fig. 2 Pictures of PI IUD (Fig. 2 A, B and C at 0, 60 and 170 hours p.c. respectively) and Cu IUD-containing horns (Fig. 2 D, E and F at 0, 60 and 170 hours p.c. respectively). The comparison is made between horns

within the same animal. In the presence of the IUD there is a reduced number of glands (cf. Fig. 2 D) and a less pronounced mucosal change (cf. A and C to Fig. 2 E and F) ($\times 10$).

ing to mucosal necrosis were seen. The number of inflammatory cells was greater in the endometrium and in the uterine lumen.

The histological picture of the endometrium described above for horns fitted with the PI IUD₁₀ was also found in horns fitted with this IUD after the treatment of the contralateral Sham-operation. Pt IUD, Cu IUD Groups (Table I).

The presence of the Pt IUD (Table I, Group II) showed a histological picture that was almost identical with the PI IUD₁₀-containing horns. However, in animals fitted with the Pt IUD, a more prominent inflammation was observed and there was an abundance of neutrophil leucocytes, mainly localized just beneath the epithelium.

The same histological changes were observed in the presence of the copper IUD both in HCG-treated (Table I, Group III) and mated animals (Table I, Group IV).

At 120 hours, the Cu IUD caused more prominent inflammation and mucosal necrosis than did the PI IUD₁₀. The appearance of the epithelium was similar in the presence of both types of IUDs, but in certain areas, in the presence of the Cu IUD, there was a thicker epithelium, sometimes with a disorganized orientation of the cells. Swollen cells were also found in these and in other areas of the endometrium. The number of glands was reduced in the presence of the Cu IUD. The glands were lined with a single-layered columnar epithelium in the presence of both types of IUDs (Fig. 1 D and G, Table I A and D). The Cu IUD evoked a more pronounced inflammatory response, and there was an abundance of both neutrophil and eosinophil leucocytes in the endometrium and in the uterine lumen.

At 72 hours, the proliferation was markedly reduced in the copper-influenced endometrium, as the number of glands was reduced, and the stratification of the epithelium was less pronounced. The minor folds were fewer and smaller, and the number of mitotic figures seemed to be reduced (Fig. 1 E and H, Fig. 2 B and E). In certain areas of the surface epithelium, some swollen cells were seen. The inflammatory response was increased in the Cu IUD-containing horn. Also at 72 hours, there was an abundance of leucocytes in the endometrium. Large vacuoles, often containing inflammatory cells, were frequently seen in the copper-influenced endometrium.

At 120 hours, the inhibition of the endometrial maturation in the presence of the Cu IUD was even more evident, as seen in a smaller number of endometrial glands and especially in a marked reduction of the development of minor mucosal folds, with fewer interconnecting bridges (Fig. 1 F and I, Fig. 2 C and F). In addition, the inflammatory response was higher in the copper-influenced horns, with a large number of leucocytes. Mucosal erosions and necroses were noted. In the copper-influenced horns, the sinusoidal character of the vessels was less evident than in the PI IUD₁₀-influenced horns.

In one group, castrated and estrogen-treated animals fitted with the PI IUD₁₀ and the Cu IUD were studied. Horns containing the PI IUD₁₀ had almost the same appearance as that found in estrus rabbits (Fig. 3 A). The major folds were lined with a high columnar epithelium, as were the normal appearing glands (Fig. 3 B). In the copper-influenced horns, the small major folds were lined with a low cuboidal epithelium, and very few glands were seen (Fig. 3 C and D). Furthermore, the diameter of these horns was markedly reduced (cf. Fig. 3 A and C). A marked inflammatory response and areas of mucosal necrosis were also seen.

DISCUSSION

The appearance of the histological picture of the endometrium in the sham-operated horns (of animals fitted with the PI IUD₁₀ in the control horn) agreed well with earlier reports on the maturation of the endometrium during early pseudopregnancy of the rabbit (11). The present study revealed no obvious differences between the appearance of the endometrium in pregnancy and in pseudopregnancy. This is somewhat at variance with the results of Beier & Kuhnel (5), who stated that the uterine epithelium underwent symplastic transformation between the 4th and 5th day in pseudopregnancy and between the 5th and 6th day in normal pregnancy. This discrepancy might be due to different strains of animals and also to the dose of HCG used (in our study 75 IU, Beier & Kuhnel's study 50 IU).

As the mere presence of an inert IUD might cause changes of the histological picture of the endometrium, the effects of the Pt IUD and the Cu IUD were compared with the effect of the PI IUD₁₀, which was always inserted into the contralateral horn.

All types of IUDs studied in the present investi-



Fig 3 Appearance of Pt IUD₁₀ (Fig 3 A and B) and Cu IUD-containing horns (Fig 3 C and D) in castrated and estrogen treated animals. The comparison is made

between horns within the same animal. There is no response to estrogen in the Cu IUD-influenced horn (Fig 3 A and C $\times 30$ Fig 3 B and D)

gation caused endometrial erosions and mucosal necrosis. These traumatic effects were more pronounced in the presence of the Pt IUD and the Cu IUD than in the presence of the Pt IUD₁₀. Earlier several authors (2, 8, 28) described such effects of IUDs in rabbits.

The presence of a copper IUD also caused a more marked inflammatory response in our rabbits than did the inert IUDs. This finding agrees well with the report by Cuadros & Hirsch (10). However, Hagenfeldt (17) described only a mild inflammatory response in human wearers of the Cu T 200 and Salaverry et al (38) found no inflammatory reaction at all.

Abraham (1) found no effect of a copper IUD on the histological picture of the rabbit endometrium

during the first four days of pregnancy. Zipper et al (47) found an intense proliferation of the rabbit mucosal stroma in the presence of the copper IUD. Zipper et al interpreted this as an increased sensitivity of the endometrium to estrogen. Acido & Zipper (3) also reported increased uptake of estrogen in the presence of the copper IUD in the rat. In contrast with the response to estrogen in castrated rabbits, the development of the endometrium was inhibited furthermore the epithelial cells of the uterine cavity were cuboidal and not of the columnar type as would be expected in an estrogen-influenced endometrium (13). In the reduced number of glands in the

nced horns of oestrus rabbits at 0 hours might a decreased responsiveness of the endometrium to endogenous estrogen. In the present study it is obvious that the IUD in contrast to inert IUDs caused a marked inhibition of the proliferation and development of the endometrium. As far as we know this effect of the copper IUD has not previously been described. In two earlier studies (15, 16) the DNA synthesis of the endometrium was reported to be significantly inhibited by the presence of the Cu which is in good agreement with the inhibition of proliferation described in this paper.

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PLASMA AND URINARY OESTRIOL DURING LATE UNCOMPLICATED PREGNANCY

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act Plasma levels of unconjugated oestriol and urinary excretion of conjugated oestriol were measured in healthy women with uncomplicated late pregnancy with normal renal function. Considerably larger differences within or between days were noted for the plasma levels than for the urinary excretion of oestriol. No significant correlation between plasma levels and urinary excretion was found in only three out of ten patients. Due to large variations within and between days the estimation of unconjugated oestriol in plasma might be unreliable as a substitute for the estimation of urinary conjugated oestriol in the supervision of complicated pregnancies.

Estimation of the urinary excretion of oestriol or oestrogens has been used for the supervision of high risk pregnancies for nearly 20 years (for a review see Frandsen 1963). Due to the variability of urinary excretion it is desirable to perform serial estimations using minimum 24-hour samples (7). However, a complete 24-hour urine collection is difficult to accomplish even in lying-in patients. Moreover, delay implicit in the collection of a 24-hour sample may cause loss of valuable time. It is one of these disadvantages that many investigators have advocated plasma analyses for the monitoring of high risk pregnancies. 90% of the oestriol in peripheral plasma is conjugated, mainly by glucuronic and sulphuric acids. Estimation of conjugated oestriol in plasma will therefore not include hydrolysis of the conjugates before analysis. This step is not included in the assay of conjugated oestriol and this assay will therefore be rapid. The purpose of the present investigation was to study the inter relationship between plasma levels of unconjugated oestriol and the urinary oestriol excretion during the last month of uncomplicated pregnancy.

MATERIALS AND METHODS

Patients

Ten healthy women with uncomplicated pregnancies were studied. All subsequently gave birth to single infants with birth weight exceeding 2500 g. 24-hour urinary specimens were collected twice a week from the 36th week of pregnancy. During each sampling day blood samples were drawn into heparinized Vacutainers® at 08.00 a.m., 08.00 p.m. and 08.00 a.m. After centrifugation the plasma was stored at -20°C until analysis.

Methods

The plasma levels of unconjugated oestriol were estimated by radioimmunoassay as described by Lindberg et al. (8). Urinary oestriol concentrations were estimated according to Frandsen (6). All oestriol assays were performed in duplicates. In order to investigate the renal function and to correct for the possibility of incomplete urine collection creatinine in plasma and urine was analysed using the Technicon method at the Department of Clinical Chemistry, University Hospital Uppsala. The osmolality of the urine was estimated by measuring the depression of the freezing point.

Statistical methods

To investigate the possibilities to predict urinary excretion of oestriol from plasma levels the coefficients of correlation were calculated between urinary excretion and each of the three blood samples. The following notations were used: n = number of sampling days; x = oestriol (ng/ml) in plasma at 08.00 a.m. the first morning of the sampling day; x_1 = oestriol (ng/ml) in plasma at 08.00 p.m.; x_2 = oestriol (ng/ml) in plasma at 08.00 a.m. the following day; y = urinary oestriol in mg/24-hour or urinary oestriol in μ g/mg of creatinine; S = S.D. of y ; r = coefficient of correlation between x and y .

In Table I the coefficients of correlation significantly different from zero are denoted by $^*P \leq 0.05$, $^{**}P \leq 0.01$ and $^{***}P \leq 0.001$.

RESULTS

The analysis of plasma and urinary creatinine revealed a normal renal function in all patients. The

Table 1 The correlation between plasma levels of oestriol (x) and the urinary excretion calculated per 24 hours and per mg creatinine respectively

The abbreviations are explained in the chapter 'statistical methods' to which the reader should refer the table. On some occasions the creatinine values in urine were not measured which explains the difference of sampling days (n) used for the statistical analysis in the two columns.

Patient	$x = \text{oestriol/24 h}$							$y = \mu\text{g oestriol/x}$			
	n	x_1	x_2	x_3	x_4	S_y	r_1	r_2	r_3	n	y_1
B	6	17.8	15.3	18.7	34.8	4.6	-0.26	0.39	0.37	6	17.8
A	8	14.8	13.4	17.7	24.5	4.8	0.55	0.67	0.47	8	14.8
D	10	21.1	21.6	20.3	23.4	5.0	0.41	0.35	-0.34	9	20.7
G	10	13.8	16.1	11.3	27.0	4.7	0.17	-0.17	-0.13	9	13.5
K	10	16.4	15.4	16.6	44.0	8.9	0.18	0.30	0.30	9	15.6
L	12	7.7	8.8	8.9	18.4	4.8	0.57	0.77	-0.78	10	6.9
M	13	11.3	12.1	12.0	28.4	4.6	-0.70	0.67	0.61	11	11.3
N	5	10.0	17.0	10.2	15.5	1.5	-0.43	0.68	0.50	5	10.0
S	8	12.3	16.5	12.3	21.9	3.5	0.48	-0.31	0.61	8	12.3
W	11	22.5	20.8	19.5	39.2	13.4	0.69	0.55	0.15	9	21.7

plasma levels of unconjugated oestriol showed considerable variations. Differences amounting to 15 ng/ml within one 24-hour period were noted on one occasion in three patients. The remaining patients displayed considerably less variation within as well as between days.

The values for the urinary oestriol excretion varied less than the plasma levels. On one occasion one patient had a difference between two days of more than 15 mg/24 hours, but in most cases the differences were less than 5 mg/24 hours.

The correlation between the total amount of oestriol per 24-hour urinary specimen and the amount of oestriol per mg of creatinine was highly significant, indicating an almost complete urine collection. The correlation between plasma oestriol levels and the 24-hour excretion of oestriol was significant only in three out of ten patients (Table 1). Two of these patients had a significant correlation between plasma concentrations of oestriol and urinary oestriol per mg of creatinine, while no such correlation could be demonstrated for the third patient. It should be noted that the coefficients of correlation have a large variation and even negative values occur. There seems to be no effect of the time of blood sampling during the day upon the correlation between the plasma and urinary values.

DISCUSSION

The concentration of unconjugated oestriol in plasma is dependent upon several factors. The rate

of production in the feto-placental unit, the transformation of the steroid in the body fluid, the transformations during the enterohepatic circulation and the conjugation capacity in liver. (For reviews of this subject see Levitz, 1970 and Diczfalussy, 1974). The excretion of oestriol is, in addition, dependent upon the renal function, as a diminished renal function will give a reduced renal clearance for oestriol in high plasma levels and a low excretion (1). However, the renal function was normal in all patients included in the present study.

The findings of large variations within two days for the plasma levels of oestriol is in accordance with previous findings in another group of healthy women (9). The fluctuations do not reach the fetal levels below 4 ng/ml reported for patients with eclampsia (10) and thus might be of physiological importance.

The lack of significant correlation between plasma concentrations of unconjugated oestriol and the urinary excretion of oestriol per mg of creatinine in the present investigation may be due to several factors. To a certain extent, the imprecision of the determination may contribute to this conclusion. The coefficient of variation for the oestriol method in these ranges was 11% for the urinary oestriol method (11). Another factor is the fact that the non-conjugated oestriol represents only 10% of the total oestriol in plasma, which is a constant production rate in the fetus.

are used. In many routine urinary oestriol methods the specificity has been deplorably neglected in order to gain marginal savings of labour.

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S	r ₁	r ₂	r ₃
3.7	-0.09	0.64	0.33
2.4	-0.03	0.60	0.39
3.8	0.48	0.36	-0.37
3.7	0.30	-0.43	-0.24
5.6	0.56	-0.58	0.40
3.7	0.75	0.88 *	0.58
1.9	-0.15	0.54	-0.10
2.7	0.70	0.79	0.43
3.0	0.68	-0.32	0.60
6.7	0.87	-0.55	-0.30

marginal changes in the rates of formation or clysis of the conjugates might cause large alterations in the unconjugated oestriol levels.

In contrary to the estimation of unconjugated oestriol in plasma, measurements of conjugated oestriol in plasma oestriol yield results with good correlation to the urinary excretion values (4, 5). Estimation of conjugated plasma oestriol has hitherto been a time consuming due to the hydrolytic step involved. However, direct radioimmunoassays on conjugated plasma using suitable antibodies against oestriol conjugates in combination with ¹²⁵I or labelled tracers might certainly appear in the future. Such a method will offer a very rapid technique for the plasma determinations and might thus substitute the urinary oestriol assay as the reliable parameter for the monitoring of fetal development. At present, however, urinary oestriol determination will still be the method of choice for this purpose, provided that proper and specific methods

ANNOUNCEMENTS

VIII Akademische Tagung deutschsprechender Hochschullehrer in der Gynäkologie und Geburtshilfe Wien 13-18 Juni 1977 Anfragen sind zu richten an Prof. Dr. H. Husslein, c/o Wiener Medizinische Akademie, Alser Straße 4, A-1090 Wien, Austria. Tel. (0222) 427165. Telegramme: Medacad Wien.

An International Conference on The Human Vagina: Biological and Clinical Aspects will be held at Wayne State University School of Medicine, Detroit, on October 28-30, 1976 (following FIGO meeting in Mexico City). Forty-five national and international experts have been invited to discuss the embryology, morphology, anatomy,

ultrastructure, cytology, physiology, bacteriology, pharmacology, immunology, microbiology, ecology of the vagina with special emphasis on human variations: intersexuality, congenital defects, secretory disorders, infectious diseases, obstetrical & non-obstetrical injuries, bacterial & viral infections, plastic surgery and vaginal contraception. Accreditation: Deadline to submit abstracts of research papers July 1, 1976. For abstract forms and additional information: Dr. E. S. E. Hoffer, Research Physiology Laboratories, C. S. Mott Center for Growth and Development, Wayne State University School of Medicine, Detroit, Michigan 48202, U.S.A. (313) 577-1011.

A NEW TECHNIQUE FOR MEASUREMENTS OF THE URETHRA PRESSURE PROFILE

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Abstract A new standardized technique for continuous recording of the urethral pressure profile simultaneously with intravesical pressure has been developed. The pressures were recorded using two micro-transducers embedded in a thin Dacron catheter. The catheter moved with constant speed through the urethra with the aid of a specially designed instrument. This instrument is described. Twenty-five healthy women were examined. The results were divided into two groups (A) 10 postmenopausal women and (B) 15 fertile women. The results of the recordings showed that the functional length and the absolute length of the urethra could be reproduced with an error of <1 mm. The maximal pressure amplitude was significantly less in group A.

INTRODUCTION

The urethra is a tubular structure only about 3 cm long in women. It can be described as a biological valve which keeps the urinary reservoir closed. If the pressure in the urethra at only one point under all conditions exceeds the bladder pressure the urine cannot escape the urethra (5). In healthy women the urine is allowed to pass merely for short periods during micturition. The lumen of the urethra then seldom exceeds 4 mm (9). The sum of the forces which keep the urethral mucous membrane in position varies from point to point throughout its length. These forces are a combination of active and passive components both in the urethra itself and in the surrounding tissues (7).

In recent years there has been an increased interest in recording the continuous urethral pressure profile (4, 6). If reliable measurements of the urethral pressure profile could be performed especially in relation to the intravesical pressure, very important information about urethral function could be obtained such as (1) the absolute length of the urethra, (2) the functional length of the urethra, and (3) the localization of that part of the urethra where the maximal intraluminal pressure is present at rest.

Previous techniques for measurements of the urethral intraluminal pressure and the urethral pressure profile have mainly been based on the use of open end fluid-filled catheter systems or fluid-filled balloon catheter systems. In both cases the catheters are connected to conventional transducers. The disadvantages of such catheter systems in measurements of the urethral pressure profile have been analyzed previously (1, 3).

We have previously described and tested a new technique for simultaneous intravesical and in-

DEFINITIONS

Urethral pressure=The pressure within the urethra recorded in relation to the atmospheric pressure.
Intravesical pressure=The pressure within the bladder recorded in relation to the atmospheric pressure.

Urethral pressure profile=Recording of the intraluminal pressure at every point throughout the absolute length of the urethra (4).

Urethral closure pressure=The intraurethral pressure minus the bladder pressure (5).

Functional length of the urethra=The distance between that point of the urethra where the intraluminal pressure exceeds the bladder pressure and that point where the intraurethral pressure equals to atmospheric pressure.

Absolute length of the urethra=The part of the urethra where the intraluminal pressure exceeds the bladder pressure.

Pressures in mmHg. **Lengths** in mm.

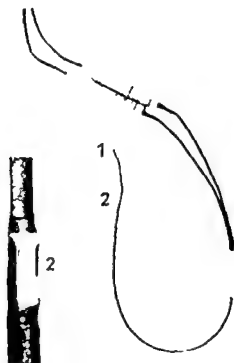


Fig 1 Catheter used for recording of the intravesical and intraurethral pressure. The pressure is recorded from the two micro-transducers nos 1 and 2 enclosed in the catheter 6 cm apart. Transducer no 2 is demonstrated enlarged six times.

traurethral pressure recording (1-3). The result of this test showed that the new technique should be excellent for continuous urethral pressure profile measurements on the condition that the catheter could be moved at a constant speed through the urethra (6). For this reason a special instrument had to be developed.

The present investigation was undertaken in order to verify this statement.

THE PRESENT TECHNIQUE

A new technique for simultaneous measurements of the intravesical and intraurethral pressure has been presented previously (1-3). In a Daeron catheter 36 cm long with an outer diameter of 4.7 F, two micro-transducers (Millar Instruments Inc, Houston, Texas, USA) were mounted 6 cm apart (Fig 1). These micro-transducers have a frequency response of more than 1000 Hz and no zero or temperature drift. The catheter was connected to an am-

1 F (French) = 0.33 mm

plifier. Not only the signals from the two transducers were amplified but also the difference between signals. A Mingograph (FMT 81, Science, Stockholm, Sweden) was used as a recorder. The instrument (Fig 2, left) was designed in order to be able to record continuously the intraluminal pressure at every point throughout the length of the urethral pressure profile. This instrument has the following parts: A mobile stand with a motor device. A motor attached to the stand is mounted to the motor which can be brought backwards at a constant speed with the aid of a steering arm. As can be seen in Fig 2 (right) there is an outer part of the steering arm on which the catheter can be mounted. The motor and the steering arm can be set in different angles as seen in Fig 2. Frequently the catheter can be placed so that the withdrawal follows the anatomy of the urethra. At the end of the withdrawal motor there is a switch which can be used for two positions: one for insertion, one for withdrawal. When the electric current is switched on, the steering arm moves with a constant speed. With the aid of the switch the withdrawal can be halted at any time. This makes it possible to record the intraluminal pressure at any point in the

Calibration and sterilization

Static and dynamic calibration as well as sterilization were performed in the calibrator unit previously described (Asmussen, Lindström & Ulmsten, 1976). The calibrator consisted of a solution of urolool (Per-Ola, Lund, Sweden) and ammonium chloride facilitating simultaneous calibration and disinfection of the catheter.

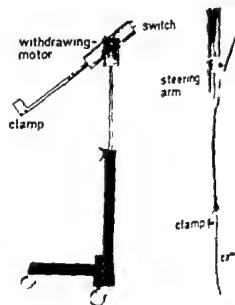


Fig 2 The withdrawal apparatus. To the left: the mobile stand with the withdrawing motor attached. To the right: the catheter mounted on the steering arm. The clamp is at the end of the steering arm.

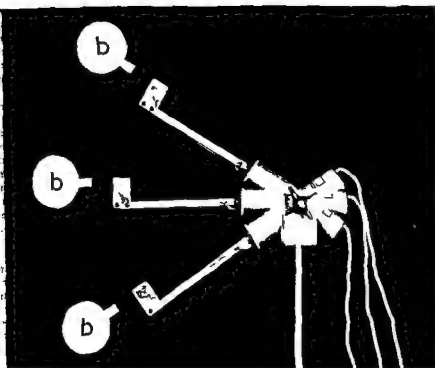


Fig. 3 The figure shows that the motor and steering arm can be placed at different angles in order to follow the direction of the urethra.

Measurement of the patients

The patient was placed in the lithotomy position. The catheter was filled with 250 cc saline. The catheter was inserted transurethraally until the two micro transducers were within the bladder and then attached to the steering of the apparatus with the aid of a clamp. To test this position the patient was requested to cough. During this it was also checked that the transducers transmitted pressure equally (Fig. 5). By starting the motor the catheter was withdrawn at a constant speed of 3.6 cm/sec. In this way the proximal transducer (Fig. 1) recorded the intraluminal pressure (Fig. 5) at every point from the internal to the external meatus of the urethra.

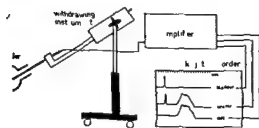


Fig. 4 The whole recording equipment

The distal transducer placed at the tip of the catheter 6 cm from the proximal transducer remained in the bladder. Consequently the intravesical and the intraurethral pressures were recorded simultaneously. Since the difference between the pressure signals from the two transducers was amplified and recorded separately on the pressure diagram, the closure pressure, i.e. the intraurethral pressure minus the bladder pressure, was obtained throughout the pressure measurement (Figs. 3, 4 and 5). With the distal transducer still in the bladder the movement of the catheter was reversed, recording another profile (Fig. 5). This procedure was easily performed without any discomfort to the patient. At least three profiles were recorded. The values in Tables I and II are mean values in mm from three consecutive measurements in each patient. Only curves with obvious artefacts due to cough or other movements were discarded. The error of a single determination (e) was calculated from the formula (10)

$$e^2 = \frac{\sum (x_i - \bar{x})^2}{n-1}$$

x_i = individual observations for person i , \bar{x} = the mean value of three determinations for a given person, n = the number of persons for whom three observations were made

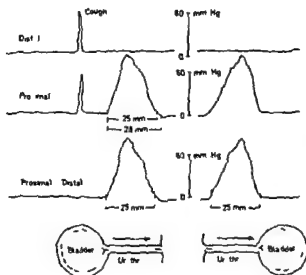


Fig. 5 Urethral pressure profile diagram from a healthy woman. The curve at the top = the bladder pressure, the curve in the middle = the urethral pressure, the bottom curve = the closure pressure (urethral pressure minus bladder pressure). To the left, both the transducers placed in the bladder. A cough indicates equal pressure transmission from both transducers. The catheter is then slowly pulled backwards. The proximal transducer no. 2 is now recording the intraurethral pressure from the inner to the outer meatus = the urethral pressure profile. The distal transducer no. 1 still in the bladder records at the same time the intravesical pressure. The maximal intraurethral pressure amplitude is in this case recorded 11 mm from the inner meatus. The functional length of the urethra is 25 mm and the absolute length of the urethra 28 mm. When the catheter was inserted, a reverse pressure profile was obtained. Note that these profiles are identical demonstrating the reproducibility of the present technique.

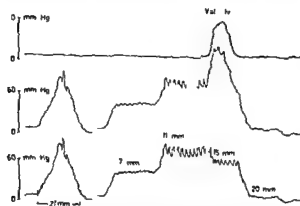


Fig. 6 In this diagram the withdrawal was stopped when the proximal transducer was 7 mm, 11 mm, 15 mm and 20 mm from the internal meatus. Note that when the proximal microtransducer was stopped at the maximal urethral pressure point of marked pulse waves were recorded. In this healthy woman a Valsalva manoeuvre did not influence the closure pressure as shown in the bottom curve.

MATERIAL

Twenty-five women volunteers were examined using the technique described above. All the patients were clinically normal. Tables I and II present data of the examination. From these tables it can be seen that the patients were divided into two groups: (A) postmenopausal women and (B) fertile women.

RESULTS

From the technical point of view, the equipment functioned satisfactorily at all the examinations. Static and dynamic calibrations before and after every examination were identical. The dissection seemed to be satisfactory and no urinary infection occurred. In all patients, urinary cultures 3 days after the examinations were negative. The relatively soft catheter was inserted through the urethra without any discomfort to the patient.

From the pressure diagram (Fig. 5) it can be seen that the intraurethral pressure was recorded

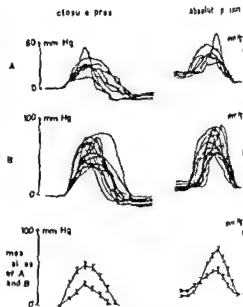


Fig. 7 This figure gives the urethral pressure profiles from each of the 25 patients examined. The uppermost part of the figure represents the 10 postmenopausal women (group A), the middle part the 15 fertile women (group B), and the lowermost part of the figure gives the mean values and the standard error of means of group A and B. The closure pressure in group A is lower than in group B. The absolute pressure in group A is lower than in group B. The closure pressure is recorded from that point in the urethra where the intraurethral pressure exceeds the intravesical pressure. The absolute pressure, however, is recorded in the atmospheric pressure.

e I Data and results of examinations in 10 healthy postmenopausal women

functional and absolute lengths the maximal urethral pressure and the bladder pressure are the means of three variations in each subject Lengths in mm Pressures in mmHg \bar{x} = mean value S.E. = standard error of the mean

nt	Age	Child births	Menopause (years)	Functional urethral length	Absolute urethral length	Maximal urethral pressure	Bladder pressure
63	0		>10	33.0	36.6	61	12
63	0		>10	24.4	30.9	31	12
69	III		>10	16.6	22.2	45	9
64	0		>10	23.0	29.6	39	10
63	II		>10	15.5	22.9	29	14
57	II		4	27.0	31.9	50	14
74	I		>10	14.7	21.8	63	17
64	II		>10	23.8	30.6	37	10
78	I		>10	23.1	31.8	40	14
58	III		8	27.6	31.8	40	10
65.3	1.4			22.9	29.0	43.6	12.2
2.1				1.0	0.9	2.1	0.8

Simultaneously with the bladder pressure. The first curve of this diagram shows the closure pressure. At the right side of Fig. 5 the reproducibility of the recording technique is demonstrated. The pressure diagram (Fig. 6) shows that the catheter can be stopped at any time during the withdrawal procedure which gives an opportunity of studying the pressure at any part of the urethra in detail over a longer period of time. As seen in Fig. 6 the catheter was stopped when the proximal transducer was in front of, at and behind the point of maximal

intraluminal pressure. Note the distinct pulse waves which had their highest amplitude at the point where the maximal urethra pressure was recorded. The length of the urethra can be calculated if the withdrawal rate and the speed of the recording paper are known. In Fig. 5 the functional length of the urethra was 25 mm and the absolute length was 28 mm. In Fig. 6 the functional length was 21 mm.

The result of the measurements of the urethra pressure profile in the 25 patients is presented in Tables I and II and Fig. 7. From these it can be

II Data and results of examinations in 15 healthy fertile women (group B)

functional and absolute lengths the maximal urethral pressure and the bladder pressure are the means of three variations in each subject Lengths in mm Pressures in mmHg \bar{x} = mean value S.E. = standard error of the mean

nt	Age	Child births	Functional urethral length	Absolute urethral length	Maximal urethral pressure	Bladder pressure
15	0		19.3	23.3	67	6
47	0		26.1	27.6	45	9
30	I		31.1	34.7	46	5
28	II		28.7	29.7	75	4
30	0		32.7	33.4	67	5
28	II		22.3	25.3	61	6
47	I		26.7	32.6	57	11
37	III		25.0	29.1	87	5
30	III		25.0	29.2	66	9
30	II		27.2	26.1	68	10
28	0		31.3	32.9	95	3
8	II		26.0	29.6	78	4
39	II		26.2	30.3	52	6
13	0		24.5	27.9	69	7
30	I		26.0	29.5	70	10
29.7	1.3		26.2	29.6	66.5	6.7
2.1			0.6	0.5	2.3	0.7

clearly seen that the postmenopausal women (group A) had a significantly lower maximal urethral pressure than the fertile women (group B). The mean maximal urethral pressure was 42.6 mmHg (group A) and 67.7 mmHg (group B) ($p < 0.001$). The elderly patients had a significantly higher bladder pressure at rest than the younger women: 12.2 mmHg and 6.7 mmHg, respectively ($P < 0.001$). No significant difference was noted in the absolute length of the urethra between the two groups: 29.0 and 29.6 mmHg, respectively. The functional length of the urethra was 22.9 mm in group B and 26.2 mm in group A. This difference is statistically significant ($P < 0.01$) but should be judged on the basis of the significantly higher intravesical pressure in group A.

The functional and absolute length showed a significant difference in each group ($P < 0.01$). The parity of the two groups was identical (Tables I and II).

DISCUSSION

As was discussed in the introduction, reliable measurements of urethral pressure can provide excellent information about the function of the urethra, especially if the intraurethral pressure is measured at the same time as the intravesical pressure (3, 5). It was stated that the following measurements of the urethra should be determined: The absolute length, the functional length, the localization and the amplitude of the maximal intraluminal pressure at rest.

The length of the urethra is considered to be of great importance in the maintenance of continence (8). Localization of the site where the maximal urethral pressure is present at rest is very important because the urethra exerts its maximal sphincter function at this point. Within this area or at this point the influence on the urethral sphincter exerted by different drugs ought to be tested. By the same token this part of the urethra should be investigated with urodynamic tests in cases of stress urinary incontinence. Furthermore, by observing the presence of pulse waves within this area, our conception of the vascular and general condition of the urethra, as well as the importance of the vascular bed for the maintenance of continence can be improved (5).

The results of the examinations in the 25 patients with the aid of our new technique showed that it

was possible to make these measurements of the urethra with a high degree of precision.

As can be seen in Tables I and II, the functional length as well as the absolute length of the urethra could be reproduced with an error of less than 1 mm ($e = 0.49$ and 0.71). From Fig. 6 it can also be seen that the maximal intraluminal pressure at rest can be determined with great accuracy both as regards the localization in the urethra and as regards the magnitude of its pressure amplitude. The pressure pulse waves had their highest amplitudes in the area of the urethra where the maximal pressure was present.

Tables I and II and Fig. 7 show that the maximal urethral pressure at rest decreased with increasing age. This observation is in accordance with that published by Enhörning (5). Urethral function is consequently also the urethral pressure profile may be impaired due to damage resulting from menopause (5, 7). However, in our investigation the parity was the same in the two groups examined (Tables I and II). Therefore, the influence of this factor on the urethral pressure profile ought to be the same in both groups of patients. Whether the decrease in estrogens in the postmenopausal women was the cause of their low maximal urethral pressure at rest (Tables I and II and Fig. 7) is yet to be proved. Preliminary results of examinations of postmenopausal women with low urethral pressure at rest more after administration of large doses of estrogens (Asmussen & Ulmsten, to be published).

The urethral pressure profile might even change during the different phases of the menstrual cycle. Therefore, we examined the fertile women 6-8 days after the first day of the last period.

The present study was carried out on a small number of patients. For this reason the final conclusions should be drawn with some caution. Our intention was mainly to perform a methodological study employing a new, clinically useful technique capable of accurate measurements of the urethral pressure profile. Evidently this technique has several advantages over previous methods: (A) it is not impaired by the risk of measurement induced in fluid-filled open-end catheter recording systems (1); (B) the frequency response of the recording system is more than 2000 Hz, which is above that needed for reliable recording of the pressure variations in the lower urinary tract. It implies a standardized, simple and reliable technique.

of the whole measuring system including simultaneous disinfection of the recording catheter. A small active measuring surface of the microtransducer (0.75 mm²) facilitates recording of the luminal pressure at practically every point in the urethra. Therefore accurate determination of the length of the urethra in addition to the localization of the maximal pressure point at rest can be performed with a high degree of precision. The intraurethral pressure is always measured simultaneously with the intravesical pressure; thus closure pressure is recorded at every phase of examination. All these advantages are obtained without any disadvantages to the patient which means that the recording catheter is used easily without any risk of discomfort to the patient. We therefore consider the present technique superior to previously used techniques for measuring the urethral pressure profile.

ACKNOWLEDGEMENTS

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ANNOUNCEMENT

Postdoctoral Fellowships in Andrology Wayne State University School of Medicine has initiated a multidisciplinary postdoctoral training program in andrology at the C. S. Mott Center for Human Growth and Development (Director: T. N. Evans, M.D.).

Trainees will conduct basic research with clinical orientation in gynecology, urology, internal medicine, pathology, immunology, or microbiology. Research interests include neuroendocrinology of reproduction, endocrine profiles, tissue culture of testicular biopsy, cytochemical pathology of sperm, ultrastructural localization of sperm enzymes, scanning electron microscopy,

immunoandrology, and bacteriology of the sperm. Fellows are expected to attend advanced courses in Human Reproductive Physiology and to participate in annual C. S. Mott Colloquia in Andrology. Selection of applicants is based on previous research experience. Double qualifications (M.D. & Ph.D. or D.V.M. & Ph.D.) are preferred. Deadline for applications is May 1, 1976. For information and application forms, write: Director of Postdoctoral Training, Dr. E. S. E. R., Reproductive Physiology Laboratories, C. S. Mott Center for Human Growth and Development, Wayne State University School of Medicine, Detroit, Michigan 48202.

SHORT COMMUNICATION

PLASMA RENIN ACTIVITY IN ABORTION

Perttu Soveri Frej Fyhrquist and Olof Widholm

From the Unit of Clinical Physiology The Minerva Institute for Medical Research (Head R. Grasbeck) Helsinki and the Second Department of Gynaecology and Obstetrics (Head O. Widholm) The University Central Hospital Helsinki Finland

Abstract Healthy women ($N=96$) were investigated for plasma renin activity (PRA) in abortion. In spontaneous abortion PRA levels were significantly lower than in induced abortion although higher than in healthy women. These findings may be explained by differences in luteal function and renin production of the fetal unit. Alternatively prostaglandins released by contracting uterus in spontaneous abortion may cause suppression as observed in 9 pregnant women with abortion induced by the intra amniotic use of prostaglandin $F_{2\alpha}$.

It is known about the role of renin in pregnancy labour in humans. It has been reported that plasma renin activity rises during pregnancy (4, 6, 14) and falls nearly to normal approximately 4 hours after parturition (1, 7, 9). It has also been shown that plasma renin concentration is raised during the first trimester of pregnancy, having a tendency to fall towards term (7, 8). Although all these factors are increased during pregnancy, the importance of this finding is not clear. To investigate the possible role of renin in pregnancy, mothers with both spontaneous and induced abortion were studied for the changes in plasma renin activity and compared with mothers with uncomplicated pregnancy.

MATERIAL AND METHODS

Sixty-six healthy pregnant women aged 17-43 years with no previous history of renal or vascular disturbances were investigated. None of them showed hypertension or retention. Twenty-six subjects had a spontaneous abortion at 9-13 weeks of gestation. The abortions were performed by evacuation and curettage. Twenty-six subjects were taken into the hospital for threatened abortion

at 8-10 weeks of gestation but did not abort and later had normal deliveries. Thirty-six subjects were normal controls at 10-40 weeks of gestation. The microscopy of the spontaneous abortions showed no histological abnormalities. All the subjects were on their normal home diet.

The blood samples for measuring plasma renin activity were collected the first day the subject came to the clinic and the day following the abortion. The control samples were taken during the subjects' normal visits to the clinic. All samples were taken after 30 minutes supine position after 12 hours of fasting at 10-11 a.m. Diet was unrestricted. Plasma renin activity was measured by a radioimmunoassay method (3, 4).

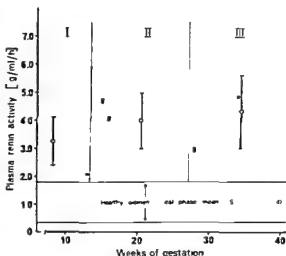


Fig. 1 Plasma renin activity in healthy pregnant women ($N=36$) throughout pregnancy. The roman numbers indicate the trimesters. For each trimester the mean \pm S.D. is indicated with open circles and bars. Reference values of healthy women in the luteal phase are also indicated.

Table I Plasma renin activity in spontaneous and induced abortion in healthy women compared to pregnancy and non pregnant women

Group investigated	A	Week of gestation	PRA mean \pm S.E.M. (ng/ml/hr)	
			Before abortion	After abortion
1 Spontaneous abortion	10	9-13	2.42 \pm 0.44	2.08 \pm 0.36
2 Induced abortion	16	8-16	4.54 \pm 0.43	5.77 \pm 0.39
3 Threatened abortion	26	8-20	4.57 \pm 0.39	
4 Normal pregnancy	70	10-70	3.86 \pm 0.74	
5 Non pregnant estrogen phase	37		0.74 \pm 0.03	
6 Non pregnant luteal phase	34		1.18 \pm 0.13	

Significances of differences obtained with Student's *t* testGroup 1 before/after $p < 0.5$ N.S.Group 2 before/after $p < 0.2$ N.S.Group 1 before/group 2 before $p < 0.01$ Group 1 after/group 2 after $p < 0.001$ Group 1 before/group 3 $p < 0.005$ Group 1 before/group 4 $p < 0.01$ Group 1 before/group 6 $p < 0.001$

RESULTS

During normal pregnancy plasma renin activity rose showing a slight increase towards term (Fig. 1). The level of plasma renin activity in subjects admitted to the clinic for induced abortion did not differ from the level in subjects coming because of threatened abortions which never occurred whereas the level of plasma renin activity in subjects with spontaneous abortion was significantly lower (Table I). Plasma renin activity before abortion did not significantly differ from the level after abortion.

DISCUSSION

The observation of raised plasma renin activity during pregnancy slightly increasing towards term agrees with previous reports (5, 7, 8, 11, 14). Plasma renin activity in subjects admitted to the clinic for induced or threatened abortion appeared to be quite comparable to the normal control material thus only showing the rise normally observed in pregnancy. On the contrary subjects having a spontaneous abortion showed a significantly lower plasma renin activity being only slightly elevated compared with healthy non pregnant women (Table I). The microscopical findings of the fetuses did not explain the abortions. The possible mechanisms could thus have been hormonal insufficiency or a blighted ovum.

Estrogens are known to increase renin substrate by stimulating synthesis in the liver (6) which has been assumed to cause the raised levels of plasma substrate in pregnancy. Progesterone is thought to

increase plasma renin activity through an aldosterone antagonizing effect (10). It is possible that an inadequate secretion of these hormones is partly the cause of the lower plasma renin activity levels observed.

Renin activity is increased in amniotic fluid and it has been shown that renin is secreted by chorionic cells of the placenta (17). It is possible therefore that part of the renin in maternal circulation is derived from the fetal-placental unit. It has been speculated (1, 8, 9) that chorionic renin is presumed to be inactive unless exposed at an early stage to pH 3 to 4 (12). In the present method for PRA measurement (3, 4) pH of plasma samples was kept at 6.0. Thus chorionic renin was not measured unless some unknown physiological activator was present. In the cases supposed to be caused by a blighted ovum the growth of the fetal-placental unit is disturbed. It can be assumed also that the capability to secrete renin is diminished.

It is difficult to state the exact time for abortion

Table II Plasma renin activity in 9 pregnant women before and after legal abortion with prostaglandin $F_{2\alpha}$ (PGF)

Sample	N	Week of gestation	PRA mean \pm S.E.M. (ng/ml/hr)
Before PGF	9	8-12	3.19 \pm 0.74
2 hours after PGF	9	8-17	1.88 \pm 0.74

Prostaglandin $F_{2\alpha}$ 50 mg was administered intramuscularly.

* Significance of the decrease obtained with Student's *t* test $p < 0.01$.

cases of spontaneous abortions. In some patients abortion had possibly taken place before admission to the hospital although the foetal unit still remained in the uterine cavity. In these cases the whole organism probably no longer acted as in pregnancy and consequently plasma renin activity was decreasing.

It could not be shown that the low plasma renin activity was responsible for spontaneous abortion. It may instead have been a sign of inappropriate reduction of estrogen and progesterone or abnormalities in the evolution of the foeto-placental unit. Low plasma renin activity during the first trimester of pregnancy seems to indicate an increased risk of spontaneous abortion.

Recently our attention was drawn to a report on decreased plasma progesterone and oestradiol levels in premature labour (13). If these observations apply to patients with spontaneous abortion and a gestation comparable to that of our patients, our observation of low plasma renin activity in spontaneous abortion cannot be explained by low production of progesterone or oestradiol.

We have recently observed suppression of plasma renin activity in abortion induced by the intra-uterine injection of prostaglandin $F_{2\alpha}$ 50 mg (ble 11). It may be assumed therefore that plasma renin activity is suppressed in spontaneous abortion by prostaglandins released by the contracting uterus into the blood stream. Suppression of plasma renin activity may offer a new basis for predicting prognosis in threatened abortion.

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CASE REPORTS

CONGENITAL ANNULAR CONSTRICTIONS DUE TO AMNIOTIC BANDS

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tract Amniotic bands which become involved with fetal parts especially the extremities are produced by rupture of the amnion during pregnancy with the consequent union of the extra embryonic mesoderm in fibrous bands. An infant with the amniotic band syndrome in which an annular constriction of the left leg and right hand is described. He was treated successfully by multiple Z-plasties and a plaster cast to correct the deformities. The oedema of the leg persisted for 5 weeks and subsided completely. The role of amniotic abnormalities in the production of congenital malformations is reappraised. The microscopic and histological investigation of the placenta and membranes should accompany every case of fetal malformation in abortuses and affected newborn.

Although congenital fetal anomalies associated with abnormalities of the fetal membranes have been described in the literature for more than 300 years, there is still some doubt that they are etiologically related (Willis 1962 Streeter 1930). According to Lippman (1968) the amnion may rupture during gestation and the extra embryonic mesoderm together with the separated amnion may form fibrous bands. Various fetal structures such as the extremities or the umbilical cord may be entangled resulting in the production of annular constrictions. If the umbilical cord is constricted fetal death will result.

The present report is of an infant with amniotic band syndrome suffering from congenital annular constrictions of the left leg and the right hand which were treated successfully.

CASE REPORT

A 22-year-old primigravida was admitted to the delivery room in her 39th week of pregnancy. Her

antenatal course had been uneventful and except for vitamin and iron supplements for prevention of anemia no medication had been administered. Her blood group was B Rh positive. On examination the abdomen corresponded to the gestational age, the fetal heart beats were normal, the cervix was dilated 2 cm and the amniotic sac was ruptured. The delivery was uneventful except for somewhat slow progress (15 hours from the time of arrival) for which an oxytocin drip was given at 7 cm dilatation of the cervix.

A male weighing 3000 g was born with an Apgar score of 10 after one minute. The placenta was expelled 3 minutes later. On examination the placenta appeared normal though a band of dense tissue was found to emerge from the fetal surface of the placenta. This band surrounded the umbilical cord near its attachment to the placenta. The histological examination of the band showed dense connective tissue which was found in some areas (Fig. 1) to be necrotic. The dense tissue was covered by amniotic epithelium.

Examination of the infant revealed a gestational age of 42 weeks. On the left lower leg immediately above the ankle a deep circular constriction was found. The skin and soft tissue was absent and the tibia and fibula were exposed. The left foot was completely flail and showed marked clubbing (Fig. 2) as well as severe oedema distal to the constriction. The skin on the leg was warm. The nail and the third phalanx of the second and the third fingers on the right hand were missing. The skin over the palm and fingers was wrinkled. Radiographic examination showed a mild deformity of the distal part of the left tibia and fibula. The distal phalanges of the second and third fingers of the right hand were absent. No other anomalies were observed.

The child was operated on at the age of one day. Under tourniquet control the medial half of the constriction was excised down to the bone and no muscles or tendons could be identified. Skin and subcutaneous tissue were sutured by multiple Z-plasties and the leg put into a plaster cast to correct the clubfoot. The wound healed satisfactorily and oedema decreased steadily. Three weeks later the remaining half of the annular constriction was operated on in a similar fashion. After excision of the constricted area a



Fig 1 A band of dense tissue emerging from the fetal surface of the placenta

considerable amount of clear oedematous fluid gushed out from the wound edges. Again, no muscles or tendons could be detected.

The postoperative course was uneventful and at the end of 2 weeks the oedema of the foot had almost completely subsided.

DISCUSSION

The formation of amniotic bands and the entanglement of various parts of the fetus, especially the extremities, with consequent developmental deformities of the fetus are ascribed to the complete or the partial separation of the amnion from the chorion. The spectrum of the deformities varies from minor defects such as syndactyly to complete amputation of a limb or even fetal death due to constriction of the umbilical cord.

According to Streeter (1930) amniotic bands are due to developmental defects occurring at the formation of the germ disc and the fetal membranes. Imperfect development leads to a localized

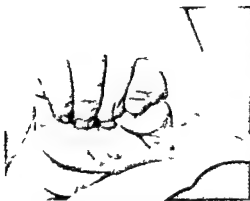
sclerosis and sloughing off the tissue which in formation of fibrous bands. Torpin (1) suggested that amniotic bands arise from rupture of the amnion without injury of the chorion. Fibrous bands are found on the detached and the chorion. Protruding fetal parts such as fingers and limbs may be entrapped by the bands and gradually become constricted and even

In addition, absorption of amniotic fluid by the amnion denuded chorion may occur and contribute to the triad of defects associated with amniotic bands: intra uterine amputations, clubbing of feet and lymphedema distal to the site of constriction. The absence of the third phalanx and third finger of the right hand only in our case may be explained by an early amputation probably due to the same cause as the annular constriction of the leg, namely amniotic bands. Syndactyly and amputations of fingers due to amniotic bands have been reported in a human fetus at an age as early as 7 weeks (Ornoy et al. 1974).

Congenital annular constrictions are currently classified into five degrees of severity. Only a shallow groove is present which requires no treatment. (1) The groove is deeper and encroaches to a varying extent on subcutaneous tissues or muscles; function is usually normal. The constricting band deepens to the bone. (2) Of varying degree is present distal to the constriction as an expression of impaired venous/lymphatic return. Sensation may be disturbed if the band is on the leg; clubfoot is usually present. (3) Symptoms are the same as in (2) but in addition pseudarthrosis of the bones tibia or fibula. (4) Intra uterine amputation.



Fig 2 A deep circular constriction is seen on the lower leg with clubbing and edema of the leg



1 The nail and the third phalanx of the second and finger of the right hand are missing

1 interesting question is how the part distal to onstriction gets its blood supply in degrees (3) (4) Stevenson (1946) postulated blood supply through the bones although it seems more probable collateral circulation is established through anastomoses of the peroneal and anterior tibial vessels which in their distal parts lie immediately over the tibia and the interosseous membrane

Several cases of amniotic band deformities have been reported in children born to mothers who have used L S D (lysergic acid diethylamide) (Zellveger 1967) Although Tjio et al (1969) could not find definite evidence to prove that L S D damages chromosomes chromosome aberrations have been described in human fetuses which have been exposed to L S D in utero

In our case a normal chromosomal karyotype 46 XX was found

1 antivitamin compound Citral when injected into a 3-day-old chick embryo was found to induce various malformations some of them associated with amniotic fibrous bands and adhesions between the amnion and the embryo (Abramovici 1967)

The teratogenic mechanism appears to be a direct effect of Citral that acts simultaneously on the embryonic tissue and the adjacent area of the amniotic membranes

1 degrees (4) and (5) of the congenital annular constrictions require early surgical treatment to improve circulation and to reduce the oedema which in later stages might become so severe as to obstruct the blood supply. The clinical features of the affected extremity including standing edema may lead to induration infection and gangrene

1 After excision of the constricted ring the skin

and soft tissue are united in zig zag fashion by rotating multiple local triangular flaps into the suture line (multiple Z plasties) thus avoiding a long straight scar which by postoperative constriction could recreate the original constriction

With constriction degrees (3) and (4) the operation is done in two stages with an interval of several weeks so as not to compromise the already precarious blood supply to the distal part. Thus the operation reduces considerably the distal oedema or even eliminates it completely. Additional orthopedic procedures are required to treat the clubfoot (casts tendon lengthening arthrodesis)

After the reduction of the oedema by Z plasties the degree of sensory disturbance usually determines the ultimate fate of the affected part since a completely anesthetic foot is prone to repeated ulceration infection and might eventually require amputation

ACKNOWLEDGEMENT

We wish to thank Dr Asher Ornoy Department of Anatomy Hebrew University Hadassa Medical School for valuable guidance and criticism and Dr L. Dolberg for radiological interpretation

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VAGINAL SURGICAL INTERVENTION FOR A SACRO COCCYGEAL TERATOMA OBSTRUCTING LABOR

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Abstract Congenital sacro-coccygeal teratomas are rare tumors and may cause dystocia. The case presented was managed unusually by incisional drainage of the tumor through the vagina in a course of an obstructed labor. Although the vaginal delivery resulted in the birth of a live baby infant.

Dystocia may be due to many maternal and fetal factors. The common fetal factors in dystocia are abnormal presentation, large size, deformities and malformations. Few cases of fetal sacro-coccygeal teratomas causing a severe degree of obstruction to vaginal delivery have been reported. In most of the cases the newborn was lost during or soon after the attempted delivery (1-6, 7). The following report is of special interest because of the unusual management applied during the course of an obstructed labor caused by a huge sacro-coccygeal teratoma and the favorable results obtained.

CASE HISTORY

The patient, Y.S., 23-year-old primigravida, was admitted to the delivery unit in active labor in her 8th month of pregnancy. The prenatal course was uneventful except for the fact that since the seventh month of pregnancy the uterus was abnormally large than was expected. On admission clinical examination of the patient estimated the fetus at term although the patient was at 35 weeks by dates. Her blood pressure was 140/80 mmHg. There was no edema of the lower extremities. The fetal heart rate was regular, 150 beats per minute. The cervix was fully effaced and dilated to 4 cm. The fetal head was at zero station. The membranes were intact. An X-ray film was done because of the suspicion of twins or hydramnios and showed a normal-looking fetus in cephalic presentation and no evidence of

cephalo-pelvic disproportion. There were no signs of hydramnios. Amniotomy was performed and labor progressed normally. A right mediolateral episiotomy was done and the head, arms and thorax were easily delivered. At this stage the delivery came to a standstill and the abdomen, buttocks and legs could not be delivered although abdominal pressure associated with traction was applied.

Palpation within the uterus revealed a very large soft tumor with broad attachment to the buttocks of the fetus. During this period the infant was breathing and grimacing. The air passages were sucked out and oxygen was given constantly to the half-born infant. The patient was immediately anaesthetized and long scissors were introduced through the vagina above the infant's back into the lower uterine segment and an incision was made on the surface of the tumor. Clear fluid was released. Immediately after this procedure and with the assistance of strong fundal pressure and firm traction of the infant's chest, a female weighing 2860 g with a partially collapsed sacro-coccygeal teratoma was extracted. The Apgar score at 1 minute was 5 and at 5 minutes 9. The third stage was normal. The incision in the tumor was sutured and the infant was transferred to the newborn unit. Under general anaesthesia examination of the uterine cavity was performed and the episiotomy was sutured.

Twelve hours later the infant was transferred to the Department of Pediatric Surgery. On examination no abnormalities were found except for the mass on the buttocks. This mass was attached to the coccyx and sacrum measured 25 × 70 × 20 cm (Fig. 1) and a large 10 cm sutured incision on the posterolateral surface could be seen. Most of it was cystic except for a thick cord attached to the vertebral column. The anal area was distorted and marked posterior compression of the recto-sigmoid colon was felt on rectal examination. Straight X-ray films of the baby revealed calcification in the mass. Barium enema showed posterior compression of the rectum and part of the sigmoid colon. On IVP moderate bilateral hydronephrosis and hydroureters with excellent excretion from both kidneys were visible. Laboratory examinations



Fig 1 Sacro-coccygeal teratoma partially collapsed immediately after delivery

revealed slight anemia and the blood urea nitrogen electrolytes liver function tests and blood gases were normal

The infant was prepared for surgical intervention during the following twelve hours with systemic antibiotics and rectal irrigations. Surgery was performed with the baby in jack knife position. Through an inverted V incision the coccyx was excised and the middle sacral vessels ligated and divided. The tumor was removed from the rectosigmoid wall and the retroperitoneal area by blunt and sharp dissection. Special attention was paid not to injure the bowel, the pudendal and sciatic nerves. A part of the gluteus maximus muscle on both sides had to be removed with the tumor. Following complete excision the wound was closed and drained. The tumor weighed 460 g and microscopic examination confirmed the diagnosis of benign sacro-coccygeal teratoma.

Postoperative recovery was uneventful and the surgical wound healed well and with a good cosmetic result (Fig 2). No neurological deficits could be detected. The baby was ready for discharge ten days postoperatively but a severe *Escherichia coli* urinary infection occurred. Aggressive antibiotic and supportive therapy together with bladder drainage at later stage could not control the infection and the baby died on the 37th postoperative day from sepsis. Permission for performing a postmortem was not granted by the family.

DISCUSSION

Congenital tumors of the sacro-coccygeal region occur every 1/35000 to 40000 births (9, 10). Sacro-coccygeal teratomas make up a small fraction of those and only a small number will cause obstruction at delivery (9). It has been estimated that dystocia may occur in 6.0 to 11.7% of cases (4, 11).

In such cases the teratomas are large at birth measuring 15–20 cm or more in diameter.

There are many theories to explain the occurrence of sacro-coccygeal teratomas. Gross et al (3) believe that they originate early in intrauterine life from pluripotent or totipotent primordial cells. They occur with greater frequency in the areas where the greatest concentration of primitive cells exist during the longest period of time, e.g. in the presomitic knot (Hensen's node) near the coccyx and reproductive gland anlage.

Sacro-coccygeal teratomas which occur predominantly in female infants (3, 4, 11) contain elements of all three germ layers, may be cystic, and are usually surrounded by a dense capsule. The potential for even apparently benign sacro-coccygeal teratomas to undergo malignant changes has been demonstrated (4). The malignant transformation occurs after 4 months of life. Total removal of the teratoma immediately after birth is necessary and the infants tolerate the operation very well (9).

Prenatal diagnosis of sacro-coccygeal teratoma is unusual. They are mostly diagnosed after birth in cases of premature delivery with small tumors when obstruction at delivery occurs (7). Plain radiographs rarely incite diagnostic suspicion but that these tumors may be present (8) if amniography is done.



Fig 2 The anal region after surgery

all tumors may be delivered vaginally by firm traction (5) but in cases of large tumors, cesareanotomy may be required (4). In most of the reported cases where obstruction at delivery occurred and operative delivery was performed either vaginally or more often abdominally, the fetus was lost during or soon after the procedure. Attempts to drain the teratomas with a needle in the course of an obstructed labor or to aspirate the tumor blindly from the fetus through the abdomen failed either because no fluid was obtained because of the high location of the tumor within the retroperitoneal cavity (1, 2, 6). In those cases it was necessary to perform hysterotomy and to excise or to remove the tumor before vaginal delivery could be attempted.

In most cases of sacro-coccygeal teratomas, the tumor is not diagnosed prior to delivery and which obstructs labor. Abdominal delivery ends in infant's death. Therefore, we would like to suggest that a large incisional drainage of the tumor should be performed to enable vaginal delivery while resuscitative measures are being taken and may lead to some successful salvage.

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LETTER TO THE EDITOR

CONGENITAL MALFORMATIONS AND DECREASED BLOOD LEVEL
OF FOLIC ACID INDUCED BY ANTIEPILEPTIC DRUGS

Sir

There are a few diseases e.g. epilepsy for which large amounts of drugs are taken. Anticonvulsive drugs cross the placenta and the fetuses of women on such therapy are exposed to these drugs during the entire gestation (1, 2).

Previous studies have shown differing degrees of teratogenicity associated with anti epileptic drugs. Speidel & Van Eck (3) found that the frequency of malformations in children born to mothers under antiepileptic treatment was eight times greater than in a control group. Speidel & Meadow (4) and Lowe (5) found a frequency of malformations 2-3 times greater than in epileptic mothers who did not take anticonvulsive drugs or in the general population. Congenital malformations are attributed to the fact that barbiturates and diphenylhydantoin decrease the blood level of folic acid by inducing an increase in microsomal enzymes which accelerate the metabolism of folic acid (6). It is known that low blood levels of folic acid in mother's blood are associated with congenital malformations and mental retardation (7).

In a retrospective study was conducted to determine the frequency of malformations among newborn infants of 70 mothers who received antiepileptic drugs during pregnancy (8). Of 56 birth children were born with malformations an incidence of 16%. Four children were born dead or shortly after delivery. Congenital heart disease, with or without cleft palate, neural tube defects and skeleton abnormalities were the commonest anomalies found.

The blood level of folic acid of two women suffering from epilepsy was measured in the seventh month of their first pregnancy. Both women had received anticonvulsant therapy for a number of years. One received trimethadione 1.2 g daily and diphenylhydantoin 0.3 g daily and primidone 1 g daily. The second received diphenylhydantoin 0.4 g daily and phenobarbital 60 mg daily. Their blood folic acid levels were 55 ng/ml whole blood and 70

ng/ml whole blood respectively (normal range 130-200 ng/ml whole blood). The first woman delivered spontaneously in the 7th month a 1700 g male infant with anencephalus, cleft lip and palate. The infant died within two hours. The second woman delivered at term a 2800 g male infant with hypoplasia.

Anti-epileptic drugs are as necessary to a mother's health during her pregnancy as they are prior to it. It is therefore recommended that women receiving anti epileptic therapy be given supplemental folic acid and their folic acid and their folic acid level monitored.

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LETTERS TO THE EDITOR

The following letter has been received from doctors Kivikoski and Pulkkinen in Turku Finland. As many of our readers probably know there is still much discussion concerning the role of progesterone for the preservation of pregnancy in women. At the Sabbatsberg Department of Obstetrics and Gynaecology we had a case two years ago where the corpus luteum of pregnancy had been extirpated on the 26th day of the menstrual cycle and the patient had a full term delivery in spite of the fact that no hormonal treatment had been given during the pregnancy.

The polemical formulation of the letter is the authors own responsibility.

Axel Ingelman Sundberg,
Chief Editor

Dear Sir

The article "Serum Levels of Oestradiol and Progesterone during Administration of Prostaglandin F_{2α} for Induction of Abortion and Labour" by O. Widholm, P. Kajanoja and E. D. B. Johansson (*Acta Obstet Gynecol* 54 135 1975) presents no new factual data and therefore deserves no comment. However the acceptance of this manuscript for publication by the *Acta Obstet Gynecol Scand* does demand considerations. For this article has been published in spite of the authors:

(1) conclusion that the action of prostaglandin F_{2α} on the myometrium does not appear to be mediated through changes in progesterone (or estrogen) levels when their own data showed statistically significant progesterone withdrawal (Pw) preceding prostaglandin (PG) induced abortion every time the number of study patients within the group was at least 10.

(2) comment that the PG provoked hormonal changes were less pronounced in the period between the 10th and 14th weeks compared with the second half of pregnancy. For their own data show the exact opposite e.g. 61% Pw at 10-14 weeks, 53% at 15-19 weeks and 26% at 36-44 weeks (all of these Pw's being statistically significant).

In view of the authors' factual data, comment is inexcusable. What makes it worse is their subsequent statement that during this period the corpus luteum is still functional and contributes to the plasma levels of progesterone. An obvious but unjustifiable effort to substantiate an earlier study of Holmdahl, Johansson & Widebäck (*Endocrinol* 67 353 1971). For the cold reality (based on 65 cases of luteectomy) that the corpus luteum becomes dispensable during the 7th to 10th week of pregnancy (Nagele) due to the luteo-placental shift in P biosynthesis (*Csapo et al. Am J Obstet Gynecol* 112 1061 1977; *115* 1973; *117* 987 1973; *Prostaglandins* 4 471 1974; *Am J Obstet Gynecol* 118 985 1974).

(3) reference to the paper of Tyack et al. as containing an argument rather than definitive supportive evidence for the earlier demonstration that hypertonic saline treatment provokes Pw before inducing clinical abortion (*Csapo et al. J Obstet Gynecol* 105 1137 1969 and *West et al. Clin Endocrinol* 30 774 1970).

(4) manipulation of historic sequence by referring to the paper of Holmdahl, Johansson and Widebäck (1971) after that of Tyack et al. (1973). In so doing the authors could say it now appears that decreased levels of progesterone found before expulsion of the fetus are not significantly related with the onset of clinical labour when the Tyack paper (written after the Holmdahl paper) brought out all the significant facts which Holmdahl et al.

(5) omission of the massive evidence that precedes PG induced abortion and that the absence of Pw signals treatment failure (*Csapo et al. Prostaglandins* 2 125 1972; *Csapo et al. J Obstet Gynecol* 103 245 1973; *Saldana et al. Prostaglandins* 1 1973; *Enkola Prostaglandins* 5 115 1974; *Enkola & Rauramo Prostaglandins* 5 269 1974; *Tiainen et al. J Obstet Gynaecol Br Comm* 81 77 1974; *Tiainen et al. Prostaglandins* 6 711 1974; *Csapa et al. Obstet Gynecol* 44 135 1974; *International Conference on Prostaglandins* Florence May 1974).

—Abstracts Craft & Youssefnejadrian Lau et al Yikorkala et al Aleem et al Schul et al Brenner et al Robbins & Mann)
 ie Editors probably adhere to the principle that content of an article published in the Journal is authors responsibility However it would now i that not all authors accept this responsibility

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ider

Sir

etter to the Editor by Drs Kivikoski and Pulk concerning our paper Serum levels of oes l and progesterone during administration of islandin F_{2a} for induction of abortion and r^m (Acta Obstet Gynecol Scand 54 135 1975) ms the old truth that when logic arguments icking emotions are used We would like to our views to the five comments made by Drs oski and Pulkkinen

Everyone working with the endocrinology of any appears to agree upon the observed mena that progesterone levels decrease be arturition This by no means implies that this sterone decrease is the driving force behind ntractions It appears more likely that it is a quence of the contractions Also in our study ostaglandin induced labour and abortions terone and oestrogen levels did decrease be at actual abortion or delivery In our opinion due to the myometrial contractions and is not ssity for the action of the administered pros dings

n order to be able to ascertain the activity of rpus luteum the only two samples that can be red are samples two and three Sample two is at the expulsion of the fetus with the placenta mple three taken 24 hours later During this eriod the contribution of the corpus r still remaining in the ovary will have a to influence the plasma level of progester asur d 24 hours after the placenta has been ed During weeks 10-14 14% of the proges level at expulsion of the fetus and placenta

was still remaining 24 hours later At 15-19 weeks 10% at 20-29 weeks 6% and at 36-44 weeks 4% was remaining These figures point to an extra pla cental contribution of progesterone during the early part of pregnancy These findings agree quite well with our previous study (Holmdahl Johansson and Wide Acta Endocrinol 67 353 1971) but they do not contradict the findings based on 65 cases of luteectomy that corpus luteum is dispensable during the 7th to 9th week of pregnancy The myometrium appears to have a low threshold for its progesterone requirements The findings that the corpus luteum can be dispensed with during early pregnancy do not indicate absence of its ability to produce and secrete progesterone Numerous reports show that in fact it appears to maintain that ability throughout pregnancy

3 We still maintain that available evidence points strongly to the release of prostaglandin by hypertonic saline infusion either by intra or extra amniotic injection (Gustavi B Acta Obstet Gynecol Scand Suppl 25 1973)

The argument under 4 and 5 is then again back to the question which comes first the egg or the hen We still maintain that there is not enough evidence to show that a progesterone decrease precedes the onset of myometrical contractions The long list of references that Kivikoski and Pulkkinen use to show that a decrease in progesterone signals failure of prostaglandin induced abortion is correct but then again high progesterone levels in this setting only indicate that the myometrial contraction has not started

Let us make one point perfectly clear The progesterone withdrawal appears to be essential in several species Even we have shown that for instance in the cow (Edqvist et al Acta Endocrinol 71 731 1972) and it may even occur in the rhesus monkey (Bosu Johansson and Gemzell Acta Endocrinol 74 743 1973) However it should be understood that the situation in women is complex and not altogether parallel to lower animals In women large amounts of progesterone circulate during pregnancy Most of it is bound to transcortin During labour a large amount of the protein bound progesterone is displaced by corticosteroids and the biologically available concentration of progesterone is likely to increase As labour proceeds the production of progesterone is impaired and also the half life of progesterone appears to be increased This is a logical consequence of the increased avail

ability of free progesterone due to the displacement from the binding sites of transcortin. The end effect is decreasing levels of total progesterone during labour.

As we regard ourselves as humble fellow scientists, history may prove us wrong. However, the other approach of strict adherence to a theory and always to interpret available facts to support this theory without close examination of other alternative possibilities may also turn out to be a mistake. We are of course quite convinced that Drs Kivikoski and Pulkkinen will accept the full responsibility of the judgement of history.

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PRENATAL DIAGNOSIS OF SOFT TISSUE MALFORMATIONS BY ULTRASOUND AND X RAY

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This study presents four cases of fetal soft malformations diagnosed during pregnancy by ultrasound scanning and amniography. The series comprises fetal teratomas, one case of fetal ascites and one of a partial mole with coexistent fetus. Diagnostic and prenatal diagnostic problems are illustrated by a fifth where the final diagnosis was a big maternal ovarian adenoma coexistent with a normal fetus.

Ultrasound scanning is a valuable part of examination of pregnant women with reference among other things to fetal malformations. For several years it has been possible to diagnose anencephaly, hydrocephaly (5, 14) and anencephaly is now demonstrated as early as in the 17th gestational week (13).

The recognition of pathologic changes in the first trimester such as missed abortion, extrauterine pregnancies and hydatidiform mole has been achieved by ultrasonic examinations (4, 7).

The diagnosis of intrauterine organic malformations is being developed but so far only a few malformations have been described (6, 9, 11).

The soft tissue masses observed by ultrasonic scanning may cause differential diagnostic problems as to their extra- or intrauterine site and also as in the last mentioned case whether they are of fetal or maternal origin. Amniography has in these cases proved out to be an essential supplement to the ultrasonic examinations (13).

METHODS

The equipment used was an Eskoline 20 ultrasonic apparatus and a Tektronix 564 storage oscilloscope. Amniography was performed by ultrasonic guidance and insertion of a puncture needle (lumbar cannula

12 mm × 15 cm) through a puncture transducer (1). As contrast Isopaque (R) 15-20 ml was used. The X-ray pictures were taken immediately after the injection and after 24 hours.

MATERIAL

The series consists of 5 patients and the indication for the ultrasonic examinations in every case was considerable enlargement of the uterus greater than expected for the gestational age.

Case I The patient a 28 year-old woman para 3 in the 19th gestational week was examined by ultrasound because of recurring vaginal bleeding. An enlarged uterus consistent with the 28th week and a living fetus in breech presentation were found. The biparietal diameter was consistent with a 19 week old pregnancy. The rest of the uterine cavity was filled by tissue with mole structure (Figs 1 and 2). Prenatal diagnosis: Partial hydatidiform mole with coexistent fetus. An induced abortion resulted in the birth of a fetus weighing 281 g. The placenta was diffusely affected by hydatidiform change and weighed 707 g. Microscopy of placenta demonstrated hydatidiform mole without malignancy.

Case II The patient a 21 year-old woman para 1 in the 33rd gestational week was examined by ultrasound because of marked hydramnios and preeclampsia. Besides hydramnios a big fluid filled cavity was found in the upper part of uterus. At first this was interpreted as a hydrocephalus but an X-ray picture showed a cephalic presentation of fetus without any sign of bony abnormality. When studying the ultrasonic pictures again it was ascertained that the fluid filled cavity described above was fetal ascites (Figs 3 and 4). The patients delivered spontaneously. The amount of amniotic fluid was 2600 ml. During the delivery the fetal abdomen was punctured and the abdominal cavity contained 1-2 l ascites. The infant was dead and weighed 3240 g. The placenta weighed 1250 g. A chromosome examination of the fetus revealed Down's syndrome.

Case III The patient a 33 year-old woman para 2 in the 25th gestational week was examined by ultrasound because of hydramnios. The uterus was found to be en-

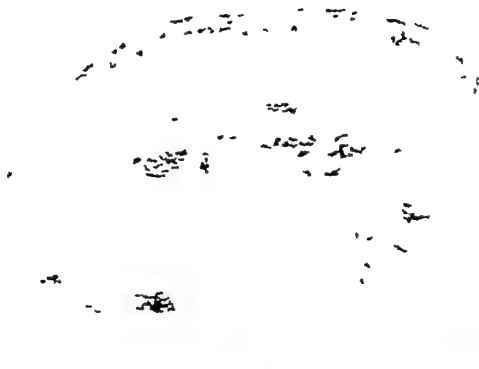


Fig 1 (case 1) Transverse scan 4 cm above the umbilicus. The fetal head (arrow) in the left side of the uterine fun-

dus. The rest of the cavity is filled by hydatidiform and hyperplastic placental tissue.

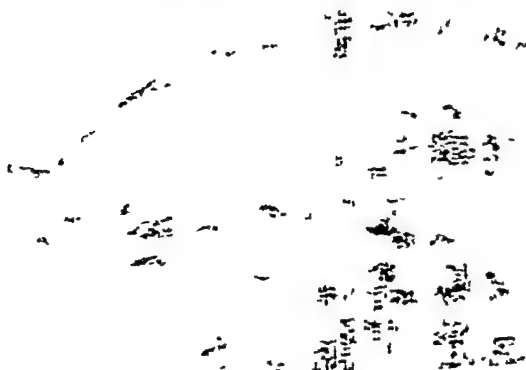
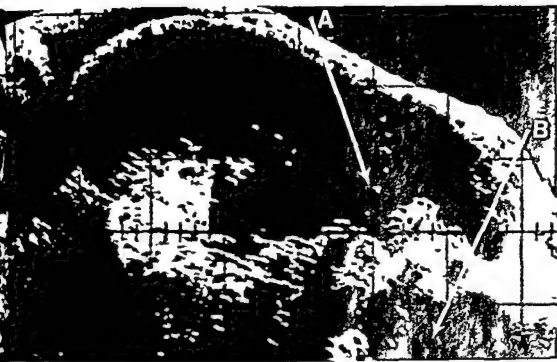


Fig 2 (case 1) Longitudinal scan at the midline. The uterine cavity is filled by hyperplastic placenta and molar tissue.



13 (case 11) Longitudinal scan to the right of the midline. The fetal abdomen is distended by ascites. A shows the fetal diaphragm. B shows the fetal head surrounded by

the arms. The head is only scanned partially because of its low presentation.



14 (case 11) Transverse scan above the umbilicus. A shows the fetal abdomen distended by ascites. B shows marked hydramnios.

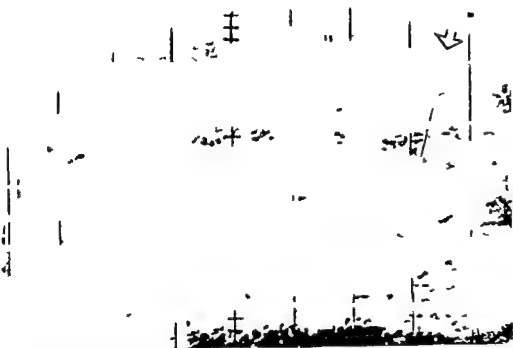


Fig 5 (case III) Longitudinal scan to the right of the mid line. A shows the head and the fetal body in a low presentation. Placenta is located at the uterine posterior wall. At

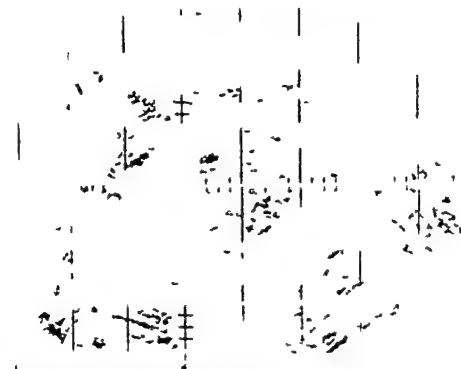
the uterine fundus (B) is seen a pathologic mass with and without echoes.



Fig 6 (case III) Transverse scan above the umbilicus. There is a pathologic mass in the uterine fundus.



Longitudinal scan to the right of the mid shows the fetal ascites. The rest of the cavity is filled by hyperplastic placental tissue and in fundus (B) is tumor like tissue.



(case IV) Transverse scan at the umbilical plane the hyperplastic placental tissue B shows the abdominal tumor tissue.



Fig 9 (case IV) The amniography shows a fetus in cephalic presentation. In the extension of the vertebral column there is a large area without any contrast (upper 3 arrows). The lower 3 arrows show the outline of the distended body.

larged consistent with the 34th week while the biparietal diameter was consistent with the gestational week. The diameter of the body was enlarged and there was hydramnios. The fundus of uterus was very difficult to scan and suggested a pathologic mass (Figs 5 and 6). The fetus died before further examinations. Induction of labor was performed. The amount of the amniotic fluid was 1600 ml.

The head was delivered the body was retained and at intrauterine palpation a tumor the size of a coconut was found. It arose from the sacral region of the fetus and was adherent to the uterine wall. The weight of the fetus was 1700 g. The autopsy demonstrated a highly differentiated teratoma arising from the fetal pelvis. The placenta was hyperplastic and weighed 1000 g.

Case IV The patient a 25 year old woman para 1 in the 26th week was examined by ultrasound because of an enlarged uterus. The fetus was a cephalic presentation with a biparietal diameter consistent with the 29th week, marked fetal ascites and an intra abdominal mass along the fetal vertebral column was found. The uterine cavity was filled by hyperplastic tissue like placental tissue (Figs 7 and 8). The fetal heart sounds were normal. The amniography pictures demonstrated a large area without any contrast in the uterine fundus in close relation to the lower part of the fetal vertebral column. The fetal body was greatly distended (Fig 9). The prenatal diagnosis was soft tissue malformation of the fetal back, ascites and placental hyperplasia. The fetal abnormalities were considered lethal and delivery was induced and was terminated vaginally with a dead fetus of 2450 g. At autopsy universal edema and 270 ml ascites were found. In the

sacral region a partially skin covered tumor was found. It weighed 512 g. The shaped one quarter of it was located with communication through the vertebral foramen. Microscopy showed a highly vascularized teratoma. The placenta weighed 1000 g.

Case V The patient a 30-year-old gestational week was examined by ultrasound an extremely enlarged uterus which had in the previous 2 months. A fetus was found in presentation without any malformations and biparietal diameter consistent with the gestational week. The fetal heart sounds were normal. A large mass surrounded the fetus on three sides, seemed edematous or vascular and whether intrauterine or extrauterine was uncertain (Figs 11 and 12). Pictures demonstrated a normal fetal skeleton. Amniography was made but the contrast was not normal area. Labour was induced and finally the infant was normal and weighed 3750 g. The placenta showed no pathologic changes. By intrauterine and intrauterine palpation immediately mass of 25x25 cm arising from the left ovary was found. The weight was 177 g. (Fig 13) a pseudomucinous cystadenoma with marked thickened wall. There was very little aqueous fluid in the

DISCUSSION

The prenatal diagnosis of congenital soft tissue malformations is important for more than

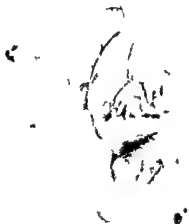
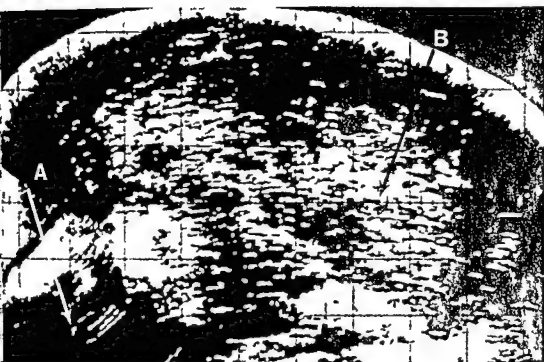
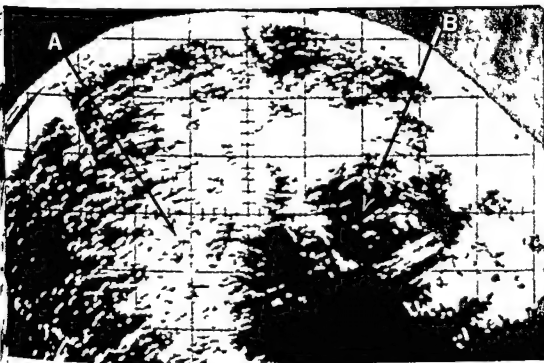


Fig 10 The fetus in case IV



11 (case V) Longitudinal scan to the right of umbili-
 A is the head B is tumor tissue



12 (case V) Transverse scan at the umbilical plane
 A is tumor tissue B is the fetal body



Fig 13 (case V) Pseudomucinous cyst adenoma

In early pregnancy so that induced abortion can be considered and later to avoid fetal and maternal complications during the delivery and if necessary in order to start treatment immediately after the delivery. If the malformation is serious the pregnancy may be terminated and thereby spare the mother some psychological trauma. If the soft tissue masses is localized outside the uterus it may affect the course of delivery and the treatment of the

A suspicion of hydramnios will often be the reason for performing examinations for possible malformations. For this purpose ultrasonic examinations are very suitable because they make it possible to diagnose not only changes on the surface of the fetus but as technique and experience improve also malformations within the fetus (6-9).

In our case (no. IV) an intraabdominal tumor was diagnosed in the fetus together with ascites. Amniography showed a distended fetal body and also a tumor situated in the uterine fundus connected with the fetal vertebral column. The amniography pictures looked like a meningocele but the ultrasonic picture showed a more solid structure like the fetal intraabdominal tumor. Case III was a fetal teratoma too in the same position as in case IV—yet the ultrasonic picture was quite different. This tumor was less vascular and less edematous. In this case amniography was not done because the fetus was dead.

Case II demonstrated like case IV the value of supplementary X ray examination. In this case amniography was unnecessary as the most important presentation on the ultrasonic scanning was discovered on the X ray picture and then the correct diagnosis could be made from the ultrasonic picture. In case V the changes were so gross that the largest part of the tumor were not included in the ultrasonic picture. When trying amniography a contrast was not found through the maternal changes. By ultrasound we got the impression that the pathology was outside the fetus and would obstruct the delivery. In case I the diagnosis of partial mole was safely made by the ultrasonic examination. This examination has so far appeared to be the most important in the diagnosis of molar disease (2, 8, 10). It has not previously been possible to diagnose a partial mole with coexistent fetus.

We have found that tissues of the same histological structure can result in different ultrasonic pictures when their liquid and solid constituents vary. On the contrary it can be difficult with ultrasound to distinguish different histological tissues if they are alike in their liquid and solid content and structure. Therefore interfaces between two such types of tissue can be difficult to scan (Fig. 8). Amniography may be valuable in distinguishing between continuous soft tissue masses within or without the uterus. Among the things which do not appear on ultrasonic pictures are the condition of the skeleton and the structure and function of the gastrointestinal tract (12, 13, 15).

Ultrasonic examinations are to be preferred at the initial examination because it is without risk and produces no side effects. The indications of making an ultrasonic scan for congenital malformations are often the same as in our cases: the suspicion of hydramnios (11), former deliveries of malformed fetuses and genetic defects in the family. It is often necessary to repeat the examination and have it supplemented by X ray pictures and amniography.

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PLACENTO THYROIDAL RELATIONSHIP IN NORMAL PREGNANCY

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Abstract Estimations of serum HCT HTSH T₄ T₃ ETR Tnosorb TBG binding capacity BMR and free total estrogen were made simultaneously in 160 women in normal pregnancy. TRH stimulation tests were made in 20 cases in each trimester of pregnancy. HCT was stable even in early pregnancy, tending to increase gradually toward the terminal stage of pregnancy as serum thyrotrophin bioactivity showed. On the other hand, serum TSH level measured by radio-immunoassay remained initially the same throughout the course of pregnancy. In the nonpregnant state, moreover, it was suggested by TRH stimulation test that pituitary TSH secreting function of pregnant women was similar to that of the nonpregnant. These findings suggest that thyroid hyperfunction during pregnancy, which is shown by progressive increase of T₃ T₄ and PBI, may not be due to high thyrotrophin but to high TBG binding capacity. Low free thyroxine may give feedback high TSH secretion but to HCT originating from placenta. In spite of thyroid hormone increase, it is true that the clinical picture of hyperthyroidism is not manifest among normal pregnant women and remained within the non-pregnant range throughout the course of pregnancy. We have also demonstrated that thyrotrophin decreased progressively. This may be interpreted to be due to the increase of TBG binding capacity. It is increased progressively and binds more of free thyroxine during pregnancy. Such a change in TBG binding capacity is well known to be caused by the effect of estrogen, which is progressively increased during pregnancy. In a word, it is possible to say that there is a placento-thyroidal system in pregnancy. HCT elevates thyroid function and TBG increased by estrogen carries thyroid hormone to target organ.

It is established that the thyroid gland becomes enlarged and hyperplastic (1, 2), thyrotoxic uptake of iodine is increased (3, 4) and the serum protein bound iodine (PBI) is elevated during pregnancy (5, 6). This may be interpreted as being due to thyroid stimulating substance that exists in the human placenta, suggested by Akasu et al (1955).

(7). Later, a purified protein with TSH activity was extracted by Hennen et al (1969) (8). Hershman et al (1971) (9) and a highly purified glycoprotein with TSH activity was extracted from the human term placenta by the authors (1972) (10). This protein hormone is called human chorionic thyrotrophin (HCT) or human chorionic thyroid stimulating hormone (HCTSH). The authors reported the biological, biochemical, immunological character of this hormone (11) and the serum HCT level was estimated by radio-immunoassay using 131I-HCT and anti HCT serum (12). In this paper, the placento-thyroidal relationship during pregnancy is discussed.

MATERIALS AND METHODS

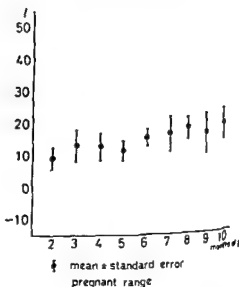
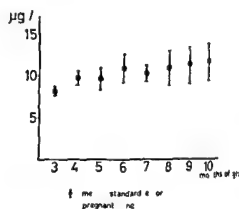
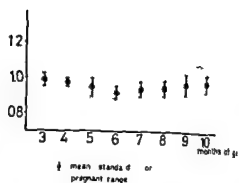
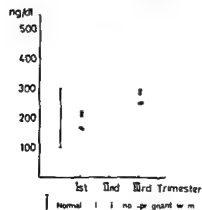
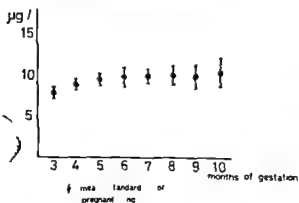
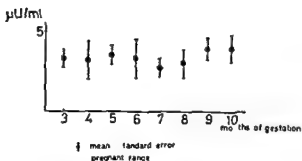
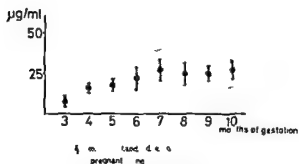
Clinical materials

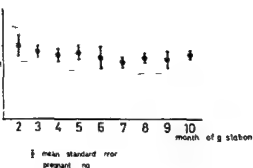
Estimations of HCT, TSH, T₃, T₄, PBI, ETR, Tnosorb, TBG binding capacity, BMR and estrogen were made simultaneously in 160 women in normal pregnancy at the Reproduction Clinic, Kobe University Hospital. TRH stimulation tests were made in 20 cases of each trimester of pregnancy. The women tested had no previous history of thyroid disease and none were on medication.

Laboratory methods

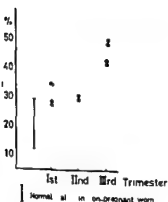
Serum HCT was measured by radioimmunoassay as described in a previous paper (12).

Serum HTSH and serum T₃ were determined by radioimmunoassay using kits of Daich Radioisotope Laboratories. Serum T₄ and effective thyroxine ratio (ETR) were measured with Res-O-Mat T₄ Kit and Res-O-Mat ETR Kit of Mallinckrodt Corporation. Serum protein bound iodine (PBI) was measured by the auto-analyzer technique and basal metabolic rate (BMR) was determined with the Sanborn-type Metabolator. Tri-iodo-L-thyronine resin sponge uptake (T₃-RSU) was measured with a Tnosorb Kit. The binding capacity of TBG was determined according to the method of Ingbar et al (13). The total estrogen





8 Triosorb during normal pregnancy



9 TBG binding capacity during normal pregnancy

was determined according to the method of Brown with some modifications. The TRH stimulation tests performed as following: 100 μ g of TRH synthesized by Dago Nutritive Chemicals Ltd. was given intravenously as a rapid injection. Serum TSH and serum HCT determined before and 10, 30, 45, 60, 90 and 120 min injection.

RESULTS

T₄ was detectable even in early pregnancy. It increased progressively during pregnancy and reached a level of 12.5–50.0 μ g/ml at term (Fig. 1). T₄ level remained within the non-pregnant range (detectable–10 μ U/ml) throughout the course of pregnancy (Fig. 2). T₄ gave a value of 7.5–12.0 μ g% in early pregnancy, 7.5–15.0 μ g% in middle pregnancy and 8.0–16.5 μ g% in late pregnancy, tending to increase within the non-pregnant range (14.0 μ g%) toward the end of pregnancy. T₃ gave a value of 150–250 ng/dl in the first, 10–320 ng/dl in the second and 210–310 ng/dl in the third trimester of pregnancy, tending to increase progressively within the non-pregnant range

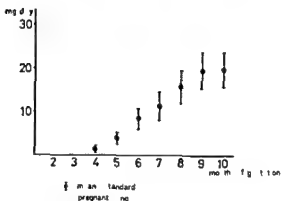


Fig. 10 Urinary total estrogen during normal pregnancy

(100–300 ng/dl) (Fig. 4). ETR remained within the range for non-pregnant status (0.8701–1.3) throughout the course of pregnancy (Fig. 5). PBI increased progressively (non-pregnant range 4.7–8.0 μ g/dl) (Fig. 6) and BMR showed a slight increase (non-pregnant range –5–+15%) (Fig. 7). Triosorb gave a value of 20–30% in early pregnancy and decreased progressively during pregnancy (non-pregnant range 26–36%) (Fig. 8). TBG binding capacity gave a value of 26–36 μ g% in the first, 28–55 μ g% in the second and 36–51 μ g% in the third trimester.

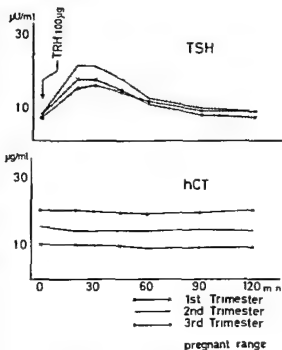


Fig. 11 TRH test in normal pregnancy

of pregnancy (Fig 9) and to be accompanied by a rise in urinary estrogen (Fig 10)

The effect of TRH on the TSH level and HCT level was studied in each trimester of pregnancy (Fig 11). A peak of TSH occurred at 20–30 min after the TRH injection with a gradual fall over the next 120 min in all cases and the level of the TSH peak was similar to that of non pregnant women. TRH did not influence the HCT level in any pregnant subject.

DISCUSSION

This paper demonstrated that the concentration of thyroid hormone increased progressively during pregnancy and it was previously reported by the authors that serum thyrotrophic bioactivity was elevated obviously and increased progressively during pregnancy.

Malkasian et al (15) and Souma et al (16) guessed that using estrogen levels caused an increase in thyroid function. They made this assumption from two well known facts: 1) The thyroid function of pregnant women is similar to that of patients receiving birth control pills or estrogen. 2) The thyroid function of the women on the pill is significantly elevated. In other words, it may be due to an over response of the thyroid caused by the elevation of TSH because of over thyroxine binding of TBG. However, the serum TSH level in pregnant women measured by radioimmunoassay is neither elevated as high as is expected from its bioactivity nor increased progressively. It remained essentially the same throughout the course of pregnancy as in the non pregnant state. Moreover, it was suggested by the TRH stimulation test that the pituitary TSH secreting function of pregnant women was similar to that of non pregnant women. For that reason, TSH does not seem to increase thyroid function in pregnancy. There must be some other explanation for the high thyrotrophic bioactivity in the serum of pregnant women and the increasing blood concentration of thyroid hormone.

It was previously suggested by Akasu et al (1955) that the human placenta might have a TSH like activity. Recently the authors succeeded in extracting a highly purified glycoprotein (HCT) from the human term placenta which has a highly specific TSH activity and they have demonstrated that immunologically it has no cross reactivity with human pituitary TSH and it is different from

other placental protein hormones such as HCS.

The authors planned to measure serum HCT by radio-immunoassay using 131I HCT and HCT. In this radio-immunoassay developed by the authors, HCT gave a value of 3.0–25.0 $\mu\text{g/ml}$ in the first 10–40.0 $\mu\text{g/ml}$ in the second and 17.3 $\mu\text{g/ml}$ in the third trimester of pregnancy. It increased gradually toward the end like thyroid hormone. These findings suggest hyperfunction of the thyroid during pregnancy may not be due to high estrogen, high T_4 , free thyroxine negative feedback, high TSH, but to HCT originating from the placenta.

In spite of the thyroid hormone increase it is that the clinical picture of hyperthyroidism is not manifest among pregnant women and TSH remained within the non pregnant range throughout the course of pregnancy and T4 decreased progressively during pregnancy. This may be interpreted as being due to the increase of TBG binding capacity which is increased progressively and more of the free thyroxine during pregnancy. A change in TBG binding capacity is well known to be caused by the effect of estrogen which is markedly increased during pregnancy (17).

From these results the authors introduce the concept of a placento-thyroidal system in pregnancy. In summary, in non pregnant women thyroid function is controlled by pituitary TSH (pituitary-thyroid system). In pregnant women it is possible that thyroid function is controlled slightly by pituitary TSH but is never elevated by pituitary TSH. Thyroid function in pregnant women is elevated by HCT itself which has a higher activity than that of TSH. Meanwhile TBG binding capacity increased by estrogen binds more thyroid hormone and keeps free thyroxine in the non pregnant range. TBG neither elevated the serum TSH level nor altered thyroid function progressively toward the end of pregnancy.

As mentioned above, it is possible to say that the thyroid gland and thyroid hormone secretion are controlled by HCT and estrogen originating from the placenta (placento-thyroidal system).

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SERUM ALKALINE PHOSPHATASE IN PREGNANCY

II Serial HSAP_{65°C} Estimations in Pregnancy Complicated with Hypertension and Pre eclampsia

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ser This is a study of heat stable alkaline phosphatase (HSAP_{65°C}) concentrations in the serum of pregnant women with hypertension (42 cases) mild pre-eclampsia (40 cases) and severe pre-eclampsia (22 cases). Results are seen in relationship to the occurrence of foetal death growth retardation intrauterine foetal asphyxia as well as the respiratory distress syndrome (RDS) in the newborn. The importance of a clinical classification of the patients is stressed. Abnormal HSAP values are those which lie either over or under the normal range for HSAP activity. In addition zig zag curves with values within the normal range are defined as abnormal. Thus serial estimations give most reliable results. Serial estimations of HSAP are really valuable in severe pre-eclampsia. Abnormal values in the 28th-38th week of pregnancy are a prognostic sign. False abnormal HSAP results found in all 3 patient groups. One possible false HSAP curve also occurred.

Placenta is an organ of high metabolic activity and contains large amounts of different enzymes. ALP is one of the enzymes of most interest—partly because of its chemico-physical characteristics and partly because it is easier to measure and separate from other enzymes of alkaline phosphatase—and partly because the occurrence of placental alkaline phosphatase (HSAP) in maternal serum can possibly be used as an indicator of placental function (1, 2, 3, 4, 5, 6, 7, 8, 9, 10).

Although the enzyme has been known since the middle of the 1930's it is only in the last 10 years that determination of HSAP in maternal serum has been used as placental function test (1, 2, 3, 4, 5). Some authors have assessed that in high risk pregnancies the activity of HSAP in maternal serum is a good indicator of placental function while others have

denied this correlation (2, 3, 4, 5, 6, 7). This disagreement can partly be explained by experimental discrepancies—for example variation in temperature at inactivation time buffer etc (2, 3, 5, 7, 10). In some studies the activity of HSAP in maternal serum is correlated with foetal weight in some with placental weight and in some with both foetal weight and foetal distress (2, 3, 5, 7, 10).

In an earlier study we determined total alkaline phosphatase (TAP) in the serum from healthy pregnant women from the 18th week of pregnancy until term. Simultaneously we did a fractionated determination of HSAP_{65°C} and HSAP_{65°C} as well as L-phenylalanine sensitive alkaline phosphatase. We found that it was only with inactivation at 65°C that the enzyme activity in maternal serum rose constantly from the 24th-25th week until term (9).

In this study we have evaluated the results of serial determination of HSAP_{65°C} in the serum of pregnant women with hypertension and pre-eclampsia. We have used serial determinations because zig zag curves are pathological even though individual values lie within the normal range (5, 6, 9, 10). The HSAP curve for each patient has been related to the baby's condition at birth as assessed by a number of clinical criteria which are related to placental function during pregnancy and/or during delivery.

MATERIAL AND METHODS

The series consists of 104 pregnant women admitted to the unit suffering from hypertension or pre-eclampsia. The classification is given in Table I. The blood pressure reading which was used for allocation of the patient to one of

Table I

Diagnosis	Features	No of patients
1 Hypertension	BP >140/90 Normal urine No oedema	42
2 Mild pre eclampsia	BP >140/90 to 160/110 Proteinuria 0.5-2% Oedema	40
3 Severe pre eclampsia	BP ≥160/110 Proteinuria ≥2 Oedema	22

Table II Serial HSAP determinations in serum from patients with hypertension and pre eclampsia

Group of patients	Normal HSAP curves	Abnormal HSAP curves	Total
Hypertension	10	32	42
Mild pre-eclampsia	7	33	40
Severe pre eclampsia	0	22	22

Table III Hypertensive and pre eclamptic women with normal HSAP curves. Fetal status

Groups of patients	Pernatal asphyxia RDS stillbirths	Weight < 2 per centile	Weight > 2 per centile	Total
Hypertension	0	1	9	10
Mild pre eclampsia	0	0	7	7
Severe pre eclampsia	0	0	0	0

the three groups was taken after at least two hours bed rest on the day of admission. Indication for delivery—the time and the method—was decided on the basis of the case history, clinical findings and other placental function tests such as oestriol estimations. The development and maturity of the fetus was assessed on the basis of serial ultra sound measurements and determination of surfactant in the amniotic fluid. The results of HSAP determinations were not included in the assessment of indication for delivery. Caesarean section was performed on fifteen women because of serious placental insufficiency. In the remaining cases contractions either started spontaneously or were induced by oxytocin before or after the expected date of delivery. Intrauterine asphyxia or prolonged second stage of labour resulted in 16 forceps deliveries and 2 vacuum extractions. Two of the vaginal deliveries were breech presentations. All the operative

deliveries resulted in live births. If the was under 37 weeks, the baby was classified as a premature. Of the premature babies there were 3 in group I (1 in group II), 3 in group II (mild pre-eclampsia) and 1 in group III (severe pre-eclampsia). Intrauterine asphyxia was diagnosed clinically or by cardiotocography. The neonatal asphyxia was applied to those babies with an Apgar score of 6 or less one or five minutes. The weight of the baby was related to a percentile worked out on the basis of the weight of all babies in Norway during the period 1967-1972 (11). for Social Medicine and Public Health, University of Bergen, Norway).

HSAP was estimated 1-2 times weekly between date of admission and delivery. Groups I and II were usually admitted so near term that tests were for only 2-3 weeks. Group III patients were admitted for several weeks and serial estimations were obtained over a longer period from these patients. Results are assessed on the basis of changes during pregnancy. Two types of pathological curves are described:

1 Curves where one or more HSAP values fall outside the normal range.

2 Curves with varying individual values which remain within the normal range (zig zag curves).

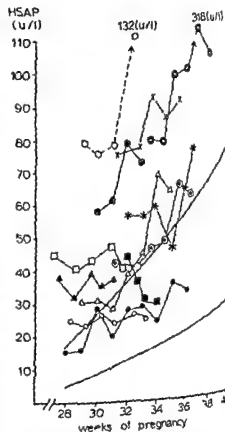


Fig 1 Serum HSAP curves from hypertensive and pre eclamptic mothers with fetal death in utero.

Table IV Hypertensive and pre-eclamptic women with abnormal HSAP curves Fetal weight distribution

Types of patients	Weight \leq 2 percentile	Weight = 3-5 percentile	Weight = 6-9 percentile	Weight \geq 10 percentile	Total
Hypertension	8	7	3	14	32
Mild pre-eclampsia	5	8	3	17	33
Severe pre-eclampsia	14	7	2	4	27

RESULTS

Patients with severe pre-eclampsia (group III) had pathological HSAP curves: some with one or two abnormally low values, some with abnormally high values and some with zig-zag values within the normal range. On the other hand, the patients with hypertension (group I) and mild pre-eclampsia (group II) had respectively 76.2% and 82.5% normal HSAP curves (Table II). Seventeen of the 27 patients had normal HSAP curves. Of these 17, 10 were in group I and 7 in group II. One of the patients in group I gave birth to a child with significant growth retardation (<2 percentile) but all others delivered healthy babies with weight >2 percentile (Table III). Pathological HSAP curves showed a close correlation with growth retardation in all 3 groups, this relation being most pronounced in group II (Table IV). Other risk factors occurred frequently in connection with pathological HSAP curves. In all cases of intrauterine death the HSAP values were abnormal, 7 in the 3rd trimester (Fig. 1). In all cases of neonatal asphyxia and RDS the HSAP curves were abnormal (Table V).

DISCUSSION

Placental function tests are of value in predicting fetal status. Placental insufficiency can result in fetal nutritional function with possible retardation

in fetal growth. The respiratory function of the placenta can also be reduced, resulting in intrauterine death, perinatal asphyxia or RDS during the first day of life. In our evaluation of the HSAP results we have taken into account all these abnormal states of the fetus and the newborn.

We do not attempt to assess the prognostic value of HSAP in relation to other placental function tests. Our aim here has been to examine to what extent serial estimations of HSAP in maternal serum mirror the function of the placenta, what limits the methods has, and what practical consequences the results may have.

In severe pre-eclampsia there is often marked destruction of placental tissue, while in transient hypertension and pre-eclampsia there is seldom such a degree of placental damage that the fetus is affected. This is reflected in our results (Table II). Mild hypertension or pre-eclampsia together with a normal HSAP curve seems to be associated with less risk to the fetus than the corresponding hypertension and pre-eclampsia combined with pathological HSAP curves (Tables III and IV). However, there was one baby with fetal growth retardation in the normal HSAP group (Table III). The baby survived and at 17 months of age it was of normal weight and development. There are many possible explanations for this, one being a false normal HSAP.

Where the HSAP curves were abnormal, there was a marked tendency to growth retardation in all

Table V Hypertensive and pre-eclamptic women with abnormal HSAP curves Fetal status
Numbers in parentheses gives simultaneous growth retardation

Types of patients	Fetal death	Perinatal asphyxia RDS	Healthy children weight \leq 2 percentile	Healthy children weight $>$ 2 percentile	Total
Hypertension	2 (2)	14 (3)	3	13	32
Mild pre-eclampsia	1 (1)	11 (7)	2	19	33
Severe pre-eclampsia	9 (9)	7 (3)	2	4	22

3 groups (Table IV) most pronounced in severe pre eclampsia. There were also more intrauterine deaths, perinatal asphyxia and RDS. Interpretation of the results is to some extent difficult since there were 10 live born premature babies and prematurity itself predisposes to RDS. In all cases of intrauterine death and several of perinatal asphyxia there was also growth retardation (Table V). Thus disasters to the fetus and the newborn may well occur in connection with pathological HSAP curves particularly in the presence of severe pre eclampsia.

In the 3 clinical patient groups there were 36 healthy babies with weight over 2 percentile even when the HSAP curves were abnormal (Table V). Other workers have demonstrated the same tendency (3). However it is worthwhile to recognize that about 60% of the newborn babies from mothers with abnormal HSAP curves had a weight less than the 10th percentile (Table IV).

Pathological HSAP concentrations in maternal serum probably imply degeneration of placental tissue. This can occur during a short period with moderate symptoms without straining the reserve capacity of the placenta.

From the findings it seems that the prognosis for the fetus is relatively good if there is no growth retardation (Table V). Pathological HSAP curves in maternal serum do not seem to indicate the danger of fetal death in the following days (Fig. 1). But severe pre-eclampsia combined with abnormal HSAP curves means that the fetus is in considerable jeopardy. Pathological HSAP curves early in

3rd trimester are a serious prognostic sign. This is in agreement with other studies (1, 6). The deciding factors are the severity and duration of the disease. From Fig. 1 it will be clear that if only a single determination of HSAP is carried out in each case it might probably be recognized as normal. However from the study of serial determinations and clinical outcome it is clear that this would have been a wrong conclusion.

It is unlikely that a single placental function test can reflect all the metabolic processes of the placenta. Serial HSAP estimations have limitations but seem to be of value. If pathological HSAP curves occur in connection with pre eclampsia termina-

tion of the pregnancy should be considered. A decision however should be taken only after studying the total clinical and laboratory picture.

The estimation of HSAP is particularly valuable while as a supplement to other placental function tests and ultra sound techniques HSAP is quick, cheap and simple to carry out.

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STUDIES IN DIABETIC PREGNANCY

I Serum Lipids

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The serum lipid values at different stages of twenty six pregnant diabetic women attending antenatal clinic at the Department of Obstetrics and Gynecology were compared with the corresponding values in four control series composed of pregnant women. Control series were studied at weeks 10, 22, 34 and after delivery respectively. Serum triglycerides were higher in the diabetic women at week 10 ($p < 0.05$) and after delivery ($p < 0.05$) than in the diabetic women. Infant birth weight was correlated ($r = 0.52$, $p = 0.05$) with maternal triglyceride values at week 31. Women with the highest serum triglyceride values (> 250 mg/100 ml) were born of infants with a higher birth weight ($p < 0.05$) than those women with lower serum triglyceride values (< 100 mg/100 ml). Intra uterine deaths ($n = 4$) were not related to maternal serum triglyceride values but mean glucose values (during the whole pregnancy) were ($p < 0.001$) in mothers with intra uterine deaths. Elevated plasma free fatty acids (FFA) in the diabetic series would be a possible cause for elevated serum triglycerides through increased liver triglyceride synthesis while in the fetus an excess of plasma FFA (passing through the placental barrier) together with normal or elevated plasma insulin would be a likely explanation for increased triglyceride synthesis in adipose tissue and possibly of increased fat depots and body weight.

In normal pregnancy maternal serum cholesterol and triglyceride values show a characteristic and massive increase toward the third trimester (4, 9). In diabetic pregnancy only limited information is available as to serum lipid and lipoprotein changes. In normal pregnancy however has long been known to be linked with high infant birth weight and furthermore this over weight of the infant has been shown to be due to an excess of fat acquired during intra uterine life. The lack of correlation between maternal blood glucose values and infant

birth weight (7) directs interest toward other metabolic variables in diabetic pregnancy.

The aim of the present study was to compare serum cholesterol and triglycerides in diabetic pregnancy with the values found in normal pregnant women. Furthermore attempts were made to relate serum lipid variables and blood glucose to perinatal data and to the fetal outcome.

MATERIALS AND METHODS

Clinical series. Twenty six consecutive pregnant women with diabetes mellitus age 19-36 years (mean 26.3) were studied at six occasions during and after pregnancy: week 10 (range 7-13), week 19 (range 18-20), week 25 (range 24-26), week 31 (range 30-32), week 37 (range 36-38) and once after delivery at day 7-5.

The women of the diabetic series attended at regular intervals a special antenatal clinic at the department of Obstetrics and Gynecology. Each woman was admitted to hospital at least once during the first and the second trimester and was kept in hospital from approximately week 30-32 until term. Each woman with diabetes required insulin treatment before pregnancy. All diabetic women were otherwise in good health and their diabetes was well-controlled. The women were grouped in relation to the degree of severity of the diabetes according to White (16) as follows: Eight women were judged as class B, eight in class C, nine in class D and one woman in class F.

Four different groups of women with normal pregnancy without any history or symptoms of metabolic disease served as a control series in the comparison of serum lipid data. The four groups of women were studied at: week 10 (range 8-12) ($n = 20$), week 22 (range 20-24) ($n = 20$), week 34 (range 30-37) ($n = 20$) and after delivery: day 2-5 ($n = 10$). In the comparison of serum lipid values data during diabetic pregnancy in week 19 and week 25 were combined designated as "week 22" and data obtained during week 31 and week 37 as "week 34".

The answers obtained at term by a questionnaire given to 112 consecutive women with normal pregnancy were

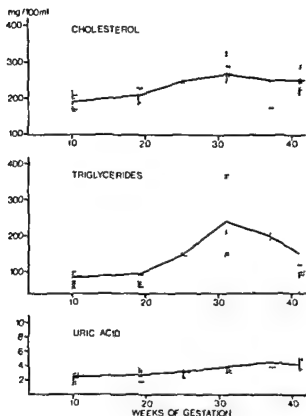


Fig 1 Diabetic pregnancy. Serum cholesterol, triglycerides and uric acid at various stages of pregnancy and after delivery. Number of subjects: week 10 $n=20$, week 19 $n=16$, week 25 $n=14$, week 31 $n=21$, week 37 $n=8$ and post partum $n=20$. Mean \pm SEM.

used in the comparison of obstetric data (control series).

Blood sampling. Blood samples were drawn from an anti-cubital vein in the fasting state in the morning after the clinical examination. After clotting the blood was centrifuged at $2500 \times g$ for ten minutes and serum was recovered.

Serum lipids and uric acid. Cholesterol and triglycerides were determined by standard automated procedures and uric acid (normal for females < 6.2 mg/100 ml) according to standard methods in the Clinical Chemistry Laboratory.

Statistical methods. Conventional methods were used for the calculation of means, standard deviations and standard error of means. Student's t test was used to evaluate differences between groups and qualitative data were compared by chi square test. Values of $p < 0.05$ were considered to be statistically significant (1).

RESULTS

Clinical data. The present series of pregnant diabetic women was characterized by a higher ($p < 0.01$) incidence (50%) of diabetes mellitus among their

relatives than women in the control series (1). Also in agreement with earlier experience in diabetic women (2, 6) these pregnant diabetic women reported a high frequency of vaginitis ($p < 0.01$) as well as of urinary infections ($p < 0.05$). There was no difference in the diabetic and control series regarding the onset of menstruation, duration of menstrual bleeding or the intervals between periods. There was a tendency to a higher incidence of spontaneous abortions in the diabetic series (11%) than in the control series (70%). Fertility was identical in the two series.

Serum lipids. Serum cholesterol and in particular serum triglycerides increased successively during pregnancy both in series of diabetic women and control series. Both serum lipids showed a maximum level in third trimester. In the diabetic series at week 31 serum cholesterol had increased compared to week 10 by 37% and serum triglycerides by 185% (Fig 1).

After delivery within 2-4 days serum lipids declined in both series (Fig 2). Serum cholesterol as well as triglycerides were, however, still higher compared to week 10 in diabetic series ($p < 0.001$ and $p < 0.001$ respectively) and in control series ($p < 0.001$ and $p < 0.001$ respectively).

Serum lipids in diabetic pregnancy as compared to the values in control series (Fig 2). There were no differences in mean serum cholesterol values between the diabetic and control series at weeks 22-34 or after delivery. Pregnant women with diabetes had, however, higher mean serum triglyceride

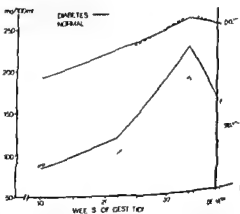


Fig 2 Diabetic ($n=26$) and normal ($n=70$) pregnant women. Mean values of serum cholesterol and triglycerides at various stages of pregnancy after delivery. $p < 0.01$.

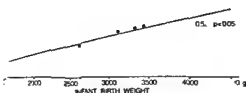


Fig. 3. Diabetic pregnancy. Relationship between maternal triglycerides (at gestational week 31) and infant birth weight. $y = 0.09x - 60.6$, $r = 0.52$, $p < 0.05$, $n = 18$.

values at week 10 ($p < 0.01$) at week 34 ($p < 0.05$) and after delivery ($p < 0.05$).

Maternal serum lipids in relation to infant birth weight (Fig. 3). In the diabetic series ($n = 18$) there was a positive correlation ($r = 0.52$, $p < 0.05$) between serum triglycerides of the mother (in week 31) and the birth weight of the infant. In the control series ($n = 20$) no such correlation between maternal serum triglycerides and infant birth weight was found.

In the diabetic series when women were divided into two groups according to their serum triglyceride values at week 31 those ($n = 9$) with serum triglycerides > 250 mg/100 ml were subsequently delivered of infants with a higher ($p < 0.05$) mean birth weight than women ($n = 7$) with serum triglycerides < 250 mg/100 ml (Table I). In these two groups of pregnant women with diabetes there was no difference between blood glucose values as determined through pregnancy (124 ± 13 and 98 ± 4 mg/100 ml respectively). These two groups did not differ in maternal age (28 ± 3 and 24 ± 1 years respectively) in gestational age in the length of the infants or the weight of the placentas (Table I).

In the diabetic series no correlation was found between maternal mean blood glucose values throughout pregnancy and infant birth weight.

Intra-uterine deaths in relation to serum lipids and blood glucose. In the diabetic series ($n = 26$) four intra-uterine deaths occurred and one mother had a spontaneous abortion in the 3rd month. The diabetic mothers with intra-uterine deaths had a higher ($p < 0.001$) mean blood glucose values throughout pregnancy than twenty-one mothers who delivered live born infants (Table II). In these series ($n = 4$ and $n = 21$ respectively) there were no differences in maternal mean triglyceride values at week 10 or in week 31.

The four mothers with intra-uterine deaths were delivered at an earlier gestational age (three infants in week 35 and one infant in week 33) than those twenty-one with infants born alive (mean gestational age 37.8 weeks).

DISCUSSION

The present series of pregnant diabetic women was characterized by a high (50%) familial incidence of diabetes mellitus and frequently a history of vaginitis (57%) and of urinary infections (46%) and also a tendency to spontaneous abortions. On the other hand the finding of normal regular menstrual periods as well as of fertility comparable to the control series was evidence that the present series of pregnant diabetic women should be considered as a representative and well-controlled group of diabetic women.

During pregnancy the diabetic women like women with normal pregnancy showed a successive increase in serum lipids, preferentially in serum triglycerides with a maximum level in the third trimester. The diabetic women however revealed even higher serum triglyceride values at week 10 ($p < 0.01$) and at week 34 ($p < 0.05$) than women in the control series. The finding in diabetic pregnancy of a marked serum triglyceride elevation in the third trimester was recently reported by Knopp et al. (8). In diabetic pregnancy also a marked increase in pre- β lipoproteins (the major triglyceride transporting lipoprotein) has been revealed by serum lipoprotein electrophoresis (11, 15). Earlier data (5, 8) would indicate however that at least in normal pregnancy an increase in serum triglycerides would only partially be due to an increase in pre- β lipoproteins (VLDL) but also be explained by a higher content of triglycerides in high density lipoproteins (HDL). Studies now in progress in

Table I. Maternal serum triglyceride values (TG) in week 31 compared with pediatric data.

Mean \pm S.E.M.

	<i>n</i>	De livery week	Infant weight (g)	Infant length (cm)	Placental weight (g)
TG>250	9	37±0.7	3781±65	50±1.4	716±53
TG<250	1	38±0.4	3108±164	49±0.8	601±32
<i>p</i> <		N.S.	0.05	N.S.	N.S.

Table II Maternal blood glucose and serum triglyceride values compared with pregnancy outcome. Mean \pm S.E.M.

	n	Blood glucose whole pregnancy (mg/100 ml)	Triglycerides whole pregnancy (mg/100 ml)	Triglycerides week 31 (mg/100 ml)
Live births	21	100 \pm 17	155 \pm 11	234 \pm 14
Intra uterine deaths	4	160 \pm 15	143 \pm 33	269 \pm 64
p <		0.001	N.S.	N.S.

normal and in diabetic pregnancy would hopefully throw further light on this question of great importance in the understanding of serum lipoprotein derangements in pregnancy.

In diabetic pregnancy the birth weight of the infant appears to be related ($r=0.52$, $p<0.05$) to elevated maternal serum triglyceride values (when triglycerides are determined in third trimester). In normal pregnancy however no correlation was found between infant birth weight and maternal serum triglycerides. Elevated pre- β lipoproteins (15) but not serum triglycerides have earlier been linked to intra uterine deaths in diabetic pregnancy. In the present study however there was no relationship between maternal serum lipid values and intra uterine deaths but mean blood glucose values were higher ($p<0.001$) in diabetic women who experienced intra uterine deaths.

In earlier studies (7) the lack of correlation between infant birth weight and maternal blood glucose values has directed the interest in this respect towards other metabolic variables in diabetic pregnancy (14). In diabetic pregnancy the serum lipid changes are also characterized by a high level of free fatty acids (FFA) (10) and recently a relationship between FFA and infant birth weight has been shown (10). Elevated serum triglycerides found in the present study as a possible indicator for infant overweight would however for practical purpose be an easier variable than FFA to measure and evaluate.

Increased serum triglycerides can either be due to an increased formation or a decreased removal (catabolism). Lipoprotein triglyceride synthesis occurs preferentially in the liver. Certain metabolic factors such as blood glucose, plasma insulin and FFA appear to influence triglyceride synthesis. An excess of FFA would enhance liver triglyceride synthesis (12) and when present simultaneously

with elevated plasma insulin and blood glucose stimulate triglyceride synthesis in the adipose tissue (3). In diabetic pregnant women elevated FFA might be a cause for increased serum triglycerides. Furthermore in the light of recent knowledge on placental transfer of FFA (16) increased FFA in the diabetic pregnant woman would be expected to cause elevated FFA in the fetus. Increased FFA simultaneously with normal or even elevated plasma insulin and access to glucose would in the fetus be a likely cause for increased triglyceride synthesis in adipose tissue. These series of events might be one possible explanation for a relationship between maternal serum triglyceride values and increased infant birth weight in diabetic pregnancy.

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INHIBITORY EFFECT OF DECIDUA ON FIBRINOLYSIS INDUCED BY UROKINASE AND BY THE FIBRINOLYTIC ACTIVITY OF THE RAT OVUM

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Abstract With the use of a technique which permits the study of fibrinolytic enzymes and inhibitors in organ culture human decidua was found to produce inhibitors capable of inhibiting plasminogen activation induced by urokinase as well as activators released from kidney explants. Also the medium in which rat and human decidua have been cultured was found to inhibit fibrinolytic activity of the rat ovum. The inhibitors emanating from the decidua are presumably of importance for facilitating the implantation of ova.

Formation of decidua is accompanied by a decrease in the fibrinolytic activity of the endometrium (1, 2). This decrease is believed to be an important factor in supporting nidation. The low fibrinolytic activity of the decidua, as determined biochemically, might depend upon a low plasminogen activator content and/or a high content of inhibitors of fibrinolysis.

Recently a method has been devised for the study of fibrinolytic activators released in organ culture.

In this method explants are cultured in the presence of but not in contact with a preformed standard fibrin clot. Fibrinolytic activators released into the medium during culture cause the gradual breakdown of the clot. Determination of the stable degradation products (FDP) accumulating in the medium provides an indirect measure of the amount of fibrinolytic activators released. The method has also been found to be suitable for the study of inhibitors released (4). Using this method we studied the inhibitory effect of human decidua on the action of urokinase and of fibrinolytic activators released by human kidney explants. We also studied the influence of inhibitors produced in

organ culture by human and rat decidua on the fibrinolytic activity of the rat ovum.

MATERIAL AND METHODS

Kidneys were taken from 16 to 18 week old fetuses obtained at legal abortion operations performed for sociomedical reasons on physically healthy women. The fetuses were removed by hysterectomy. The decidua was taken at the same time from a site opposite to the placenta in the uterus.

The tissue fragments were washed in Parker 199 (SBL, Stockholm) culture medium and divided into pieces about 1 mm across. These explants were then placed on slices of gel foam (Spongostan, Ferrosan, Malmö) four explants per slice. The slices of gel foam with the explants were transferred to Leighton tubes (two slices in each tube) containing a clot formed by addition of 0.04 ml of thrombin (Topostaslin, Roche) diluted to 75 NIH U/ml in Parker 199 to 1 ml of a solution of one gram of fibrinogen in 80 ml of distilled water and 0 ml of Parker 199. One millilitre of the culture medium Parker 199 was then added. Care was taken to avoid contact between the sponge slices and the preformed clot. In the experiments performed to assess the inhibitory effect of decidua on urokinase decidua explants were cultured in medium containing respectively 3.0, 1.5, 0.75 Ploug units of urokinase/ml (Lovens, Copenhagen). Each Leighton tube contained two slices of gel foam with four decidua explants on each. For comparison the same test system was incubated with the same amounts of urokinase but without decidua explants.

In the combined cultures two explants of decidua and two explants of kidney were placed on each slice of gel foam. Gel foam slices without explants placed in similarly prepared Leighton tubes served as controls.

After culture the kidney explants were examined for fibrinolytic activity with Todd's fibrin slide method (5) as modified by Pandolfi (6).

For determination of FDP 0.06 ml of culture medium

Table I Inhibition of urokinase by decidua in organ culture. Values given for two cultures. Each value denotes FDP (mg/ml of Parker medium)

	Days of culture					
	I		II		III	
Urokinase 3.0 U/ml	150	156	216	240	448	500
+Decidua	12	18	62	76	110	130
Urokinase 1.5 U/ml	25	30	75	100	180	210
+Decidua	7	7	19	25	32	45
Urokinase 0.75 U/ml	15	2	13	17	50	70
+Decidua	0	0	0	1	4	6
Decidua	0	0	0	0	15	2
Control	0	0	0	0	0	1

was collected every 24 hours with a capillary pipette. FDP were determined with a quantitative immunological method (7).

An experiment was performed in which human decidua as well as rat decidua was first cultured as organ culture for three days in Parker medium without a preformed clot. Both culture media were then examined for their ability to inhibit fibrinolytic activity of the rat ova in the following way: glass slides were prepared covered with a fibrin film. This film was obtained by mixing 0.06 ml of fibrinogen (bovine fibrinogen prepared essentially according to Brakman's modification (8) of Astrup and Möllertz's (9) double ammonium sulphate precipitation method in a concentration of 1% in phosphate buffer pH 7.8 ionic strength 0.15) and of 10 μ l thrombin (Topostasin 20 NIH U/ml unbuffered saline). The fibrinogen-thrombin mixture was spread over an area of 10 cm² in order to obtain a film about 0.07 mm thick. To stabilize the fibrin film, slides were left at room temperature (21–24 °C) in a moist chamber for 30 minutes.

To some of the slides decidua culture medium and to some serving as controls Parker medium 199 not previously used for culture was added to the thrombin-fibrinogen mixture to a final concentration of 40%. Rat ova obtained by flushing the fallopian tubes on day 2 of pregnancy and washed in physiological saline were transferred to the fibrin film with a micropipette. The slides were then incubated in a thermostatically controlled (23 °C) moist chamber for 30 minutes. The lytic area in the fibrin film was then calculated in square microns of lysis. Fifteen ova were examined on each kind of slide prepared.

RESULTS

The inhibitory effect of decidua explants on the activation of plasminogen by urokinase is apparent from Table I which shows a marked inhibition in all the concentrations used. In the concentration of 0.75 Ploug units per ml of medium the inhibition

was almost complete. There was a corresponding release of fibrinolytic enzyme from the renal explants as measured by the increase in FDP in the culture medium. When the renal explants were cultured together with decidua explants the amount of FDP was much smaller (Table II).

In the controls and the cultures of decidua alone FDP were barely detectable on the third day.

The fibrinolytic activity of the rat ova in the medium on fibrin slides without culture medium or with addition of fresh medium was found to be $90 \pm 10 [\times 10^3]$ square microns. On the slides to which human decidua culture medium or that of decidua was added to the fibrin film the fibrinolytic activity never exceeded the area of the ovum, i.e. $< 18 \times 10^3$ square microns.

An additional experiment was performed in which decidua and human fetal lung tissue were first cultured as organ cultures for two days in Parker medium without a preformed clot. The medium from these cultures was then used as culture medium for kidney cultures in Leighton tubes with a preformed clot and also examined for its ability to inhibit urokinase. We found that the medium from decidua cultures but not from lung cultures inhibited activation of plasminogen by urokinase and the enzymes liberated from the kidney explants. Histochemical examination of the cultured kidney explants invariably revealed persistent fibrinolytic activity.

DISCUSSION

The results show that human decidua in organ culture liberates agents capable of inhibiting the activation of plasminogen by urokinase as well as the fibrinolytic activators released by kidney explants. That the inhibitory effect of the decidua

Table II Inhibitory effect of decidua on fibrinolytic activity in kidney organ cultures

Values given for two cultures. Each value denotes FDP (mg/ml of Parker medium)

	Days of culture					
	I		II		III	
Kidney	8	37	125	168	400	484
Kidney+Decidua	0	3	4	9	9	14
Decidua	0	0	0	0	0.5	1
Control	0	0	0	0	0	1

be ascribed to adsorption of the activators to the surface of the decidua explants is apparent from the control experiment in which the medium from decidua culture had an inhibitory effect while medium from lung tissue had not. The persisting fibrinolytic activity found in the kidney explants suggests that the inhibitory action takes place in the decidua.

The results also demonstrate that the inhibitors released by human as well as by rat decidua explants in organ culture are capable of inhibiting the fibrinolytic activity of the rat ovum. It has been known recently that rat ovum possess a high fibrinolytic activity during their passage through the fallopian tube. This activity decreases when the ovum leaves the uterus and disappears at implantation.

During the formation of decidua the fibrinolytic activity of the endometrium decreases (1, 2). It has been assumed that the decrease in the fibrinolytic activity of the endometrium and the disappearance of the fibrinolytic activity of the ovum is of importance for implantation. This is analogous to the situation in organ culture on clotted substrates. When fibrinolytic active explants are placed on clot substrates they dissolve the substrate and become detached (11, 12, 13). The addition of an inhibitor of fibrinolysis prevents this and promotes adhesion and growth (13, 14, 15). Judging from the present observations, inhibitors derived from the decidua are capable of inhibiting not only fibrinolysis induced by urokinase but also the fibrinolytic activity of the rat ovum. This suggests that the decidua inhibitors are of importance for facilitating implantation of the ovum.

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A PROSPECTIVE STUDY OF DRUGS AND PREGNANCY

3 Hormones

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Results: Results from a prospective study in Malmö formed in 1963-65 are used to discuss the possible role of hormonal drugs in human fetal maldevelopment. Approximately 10% of all women that had a child born were treated with gestagens during early pregnancy and slightly more used Primodos as a pregnancy test. No harmful effect on embryonic development can be demonstrated.

This is the third report on a linkage between drug use and pregnancy outcome based on a prospective study carried out in Malmö during 1963-65. Previous reports (10, 11) dealt with two groups of drugs which are often used during early pregnancy: psychopharmaca and antiemetic drugs. The need to regulate drug usage against the variability in incidence and severity of symptoms at different outcomes of pregnancy was stressed. This is perhaps more evident for one important group of drugs which has been regarded as potentially teratogenic: hormone preparations. Hormones are often given in order to adjust supposed abnormalities in the hormone situation during the pregnancy which may be caused by disturbances in fetal development.

Hormones given to the mother can undoubtedly affect the embryo. The masculinizing effect of certain gestagens on female fetuses is perhaps the known example. This is described both from animal experiments and from clinical experience (7, 10). During recent years the relationship between the use of diethylstilboestrol during pregnancy and the development in the offspring of genital dysplasias and carcinoma during adolescence has been much discussed (cf. 6). Some authors have also suggested that the use of sex steroids during early pregnancy can cause other

types of malformations, notably closure defects of the central nervous system (4, 5—contradicted by 12, 13, 19), congenital heart defects consisting of transposition of the great vessels (14, 16, 18) and hypospadias (1, 2). Special interest was then paid to hormones used as pregnancy tests, most popular before the immunological pregnancy tests had come into common use. It was recently suggested (8) that a relationship exists between oral contraceptive failure or other sex steroid treatment during early pregnancy and the birth of infants with congenital limb reduction defects. One hundred and eight women gave birth to such infants—six had become pregnant in spite of oral contraceptives and nine had had other sex steroid treatments as a supportive measure (six) or as a contraceptive accidentally started after conception (three). Among 108 mothers of normal infants, one had a contraceptive pill failure and three had received sex steroid therapy during early pregnancy.

MATERIAL AND METHODS

The information on which this study is based was collected in a prospective study of 6376 pregnancies described in some detail in the first paper of this series (10).

RESULTS

A Gestagens

Fig. 1 compares the use of gestagens during the different months of pregnancy in two groups of pregnant women: those who will later have a miscarriage and those who will have an infant born. In the latter group of 5753 women, the highest incidence of gestagen exposure was found in the third gestational month: 103 women=1.8% then had

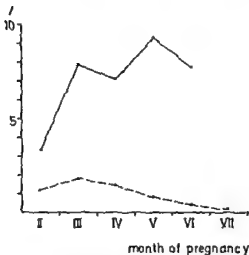


Fig 1 Percentage of women using gestagen preparations during different months of pregnancy. Comparison between women who will miscarry (—) and women who will have an infant (---)

been given such drugs. Among these 92 women had an infant that did not survive—none of the women had been given gestagens during the third month. 194 infants had major malformations. 3 (=1.5%) of the mothers had been given gestagens during the third month.

When the group of women who will later miscarry is compared with the above-mentioned group the gestagen usage is obviously much higher (Fig 1). During the third month 24 of the 305 women who later aborted were taking gestagens ($\chi^2=52.1$ at 1 d.f. $P<0.001$).

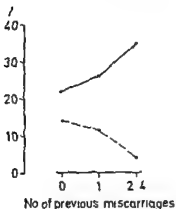


Fig 2 Percentage of women who miscarry according to number of previous miscarriages. Comparison of women who reported early bleeding during the present pregnancy (—) and women who did not report such bleeding (---). All women were given gestagens during the first trimester.



Fig 3 Percentage of women who miscarried compared between women with and without gestagen therapy. Hatched column marks women who reported early bleeding during the present pregnancy; empty column marks women who did not report such bleeding during the present pregnancy.

The obvious explanation for this is that gestagens were given in an effort to prevent a threatened abortion by correcting a supposed progesterone deficiency. Among 200 pregnancies in which gestagens were administered bleeding had occurred previously in that pregnancy in 81 and there had been a previous miscarriage in 103 (in these more than one previous miscarriage). But previous miscarriage and bleeding during the present pregnancy had occurred in 36 cases. In the remaining 50 instances the reason for gestagen administration is not clear.

Fig 2 shows the risk of miscarriage in a pregnancy treated with gestagens according to early bleeding during the pregnancy and according to previous miscarriages. The Figure shows that early bleeding is the more important prognostic factor. A previous miscarriage only increases the risk in the presence of bleeding during the present pregnancy. Fig 3 compares the miscarriage rates with and without gestagen treatment in the present pregnancy, according to whether there was or was not previous bleeding during the pregnancy. The higher miscarriage rate in the gestagen treatment group is probably because this group was selected as a high risk group. If this risk is to some extent reduced by giving gestagens cannot be decided from these figures.

Among 5002 women who gave birth to no malformed infants 98 had been treated with gestagens at some time during the first trimester, i.e. approx 2%. Among 551 women whose infants had only minor malformations 9 had been treated with gestagens, slightly below 2%. Among 145 women who had infants with major malformations

Table 1 Summary of pregnancies where Primodos used during the second month of amenorrhoea pregnancy diagnosis test

Pregnancy outcome	Total no of women	No using Primodos	% using Primodos
Survived	448	15	3.3
Induced abortion	154	13	8.4
Normal infant	5 753	128	2.2
Abnormal infant	4 910	107	2.2
Infant	97	1	—
Major malformation	551	16	2.9
Minor malformation	194	4	2.1

15 infants had hypospadias and 11 had major malformations of the genitalia but none of these mothers had used Primodos.

There was no association between gestagen treatment and gestational age at birth or weight at birth. Five of the non malformed infants of women treated with gestagens were born more than 2 weeks before the expected date compared with the expected number of seven. Three were born more than two weeks after the due date whereas 3 to 4 weeks before the expected date. Among the infants born between 38 and 42 weeks of pregnancy the weight distribution was in the expected range: less than 2.5 kg (expected 1.9) 2.5–3 kg 8 (10.2) 3–3.5 kg 36 (expected 1.9) 3.5–4 kg 29 (31.5) over 4 kg 29 (31.5).

B Primodos

Primodos is a drug containing norethisterone and ethinylestradiol and was much used as a means of inducing pregnancy. In the present series it was given to 156 women during the second month of pregnancy—most of them were given it to diagnose pregnancy. Table I presents the outcome of the pregnancies in relation to Primodos usage. It is obvious that the group of induced abortions containing Primodos shows a high incidence of induced pregnancies ($\chi^2 = 12.9$ at 1 d.f. $P < 0.001$). In the group of future miscarriages a slightly increased incidence is also seen—this agrees with the high frequency of unwanted pregnancies in this group possibly hiding a number of induced abortions (cf

C Other hormones

Gestagens and anabolic steroids were given to 65 women during the second month of pregnancy and

corticosteroids to 11. A hormone of any type was given during the first trimester to 238 of the 6376 women included in the present study i.e. 3.7%. The only hormone where an obvious association with pregnancy aberrations could be demonstrated was insulin. Among 15 diabetic women who were on insulin two had a miscarriage six had a normal living infant three had normal infants that died perinatally one had an infant with a minor malformation and three had infants with major malformations (one with gut atresia two with multiple malformations).

DISCUSSION

In spite of the possible deleterious effects on the fetus of certain hormones administered during pregnancy 3.7% of the women included in the present study reported that they had used hormonal drugs during the first trimester including the organogenetic period. One large group of them were treated with gestagens mainly in order to prevent a threatening abortion. No effect was obvious from this point of view which agrees with the now prevailing view on the lack of effect of gestagen therapy for this purpose. In a double blind well controlled study of the effect of gestagen in threatened abortion in a series of 260 cases no difference was found in the outcome of the pregnancy between patients treated with gestagen and patients treated with placebo (3).

No teratogenic effect of the gestagens could be demonstrated. It is of a special interest that none of the 15 women who gave birth to infants with hypospadias had used gestagen preparations—thus none of the 112 infants whose mothers got gestagens had hypospadias. This does not exclude a teratogenic effect of gestagens general or specific for hypospadias but if such an effect exists it must be relatively weak.

The same holds true for the hormonal preparations used for pregnancy diagnosis in our study illustrated by Primodos. None of the 128 women who used Primodos during the second month of pregnancy had an infant with hypospadias and the number of infants with major malformations in general was not increased in this group. The figures do not exclude a teratogenic effect of such hormone preparations but they give no support for it.

The main argument for an association between the use of gestagens and the development of

hypospadias is the work presented by Aarskog. In his first report (1) four women among 80 who gave birth to an infant with hypospadias had received gestagens and another woman had used Primodos. In his second report (2) the series had been increased by 20 to a total of 100. One further Primodos user was then included and three further women who had received gestagen therapy. The sampling principles for the extended series are not clear but it is noteworthy that the incidence of hormone users is higher in that series than in the first reported series (4/20-5/80). No control series was presented in any of the studies so it is not possible to know if the incidence of hormone users described is higher than that expected. Local variations are probably large both with regard to gestagen usage for threatening abortion and to the use of hormonal pregnancy diagnosis tests. In the present study approximately 2% of all women who later had an infant had taken Primodos. A corresponding figure given from Atlanta (17) is 10%.

A weak correlation can be found between the use of sex steroids and the birth of malformed infants without any cause and effect existing. Gestagens were in the present study prescribed more often if bleeding had occurred early during the present pregnancy or if previous reproductive failure had occurred than if no such complicating factor existed. Both these problems are associated with the birth of more malformed infants than normal.

The only endocrine treatment which showed a clear cut correlation with pregnancy outcome is insulin.

This is obviously due to the diabetes and not to the drug. The pregnancy wastage recorded in this group is high. According to Möller (15) the perinatal mortality should not be much increased among infants of diabetic mothers if the latter are supervised closely from early pregnancy and if their blood sugar levels are carefully controlled.

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PHOSPHOLIPID CONCENTRATIONS IN AMNIOTIC FLUID FROM DIABETIC PREGNANT WOMEN

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Amniotic fluid phospholipid concentrations were determined in 104 samples from 65 diabetic patients have related to gestational age, the state of diabetes according to White's classification and the development of respiratory distress syndrome (RDS). There is statistically significant evidence of accelerated surfactant production in White classes D-F from 34 to 37 weeks gestation. Classes A-B-C have phospholipid concentrations significantly different from a reference series (RDS treated in 6th and was frequently associated with malphospholipid concentrations). There was a significant relation between low Apgar scores and subsequent development of RDS.

It has long been recognized that newborn infants of mothers with diabetes mellitus have a higher incidence of RDS than infants of non-diabetics of the same gestational age despite a high birthweight (2). Several authors have proposed this to be due to delayed pulmonary maturation as measured by a fall in the rise of phospholipid concentrations in amniotic fluid from 34 weeks gestation (8, 10, 14). Gluck (8) has found that in the diabetic state there is a retardation of surfactant development in White's classes A-B-C and accelerated maturation in classes D-F. Several reports have indicated that lecithin/sphingomyelin (L/S) ratio determinations may be of doubtful prognostic value in diabetic pregnancies. This is based on the occasional development of RDS despite mature L/S ratios (4, 6, 2, 16, 18).

The purpose of the present study has been to investigate the maturation of the fetal pulmonary surfactant system in diabetic pregnancies as measured by the amniotic fluid lecithin concentrations and L/S ratios and to examine the relationship between these parameters and the development of RDS. Certain other factors which might influence

the surfactant system and respiratory function have also been studied.

MATERIAL AND METHODS

109 samples of amniotic fluid from 67 diabetic pregnant women were collected by transabdominal amniocentesis or at the time of caesarean section during a three year period from 1971-74. Antenatal care during the last trimester and delivery took place at the Department of Obstetrics and Gynaecology Rikshospitalet. All neonates were immediately transferred to the Department of Pediatrics. Two samples from a child with multiple malformations who died during labor were excluded together with three samples from one patient with unknown gestational age so that 104 samples were left for analysis. 94 samples were analyzed by the method previously published by Lindback et al. who had used uncentrifuged amniotic fluid which gives higher lecithin concentrations and L/S ratios (10, 11). The lecithin concentrations are given as micromoles in 100 ml amniotic fluid. 59 samples were analyzed by Gluck's method as follows: Centrifugation of the amniotic fluid at 1050 g x 5 minutes. Lipid extraction in methanol/chloroform 2:2:4. The chloroform phase was dried under N₂ followed by cold acetone precipitation, this layer chromatography with silica gel H with 5% ammonium sulphate, charring and chromoscanning. 49 samples were analyzed by both methods.

Gestational age has in the text been given in terms of weeks meaning completed weeks, i.e. 39 weeks = 273-799 days. The phospholipid concentrations from the diabetic pregnancies have been compared at weekly intervals with a reference series consisting of rhesus immunized women with rhesus negative babies or with no pathological hemolysis (i.e. Liley Zone 1) and patients with normal pregnancies. For the patients included in the reference series there should be no pathological factors influencing the surfactant production.

The Wilcoxon two-sample test, the Wilcoxon van Elteren test and the Fisher Irwin test have been used for statistical analysis. The significances given (P) are for two-sided tests.

Table I Lecithin concentrations in $\mu\text{M}/100$ ml amniotic fluid and lecithin/sphingomyelin (L/S) ratio in maternal diabetes of classes ABC and DF compared with non diabetic controls at different gestational ages

Gestational age in weeks (days)	ABC (Lecithin)	DF (Lecithin)	Control (Lecithin)	ABC (L/S ratio)	DF (L/S ratio)	Control (L/S ratio)
<34	4.37	0.67	0.51	6.94	2.03	0.61
<238		1.69	0.56		3.19	1.22
		2.47	0.70		3.80	1.77
			0.89			1.44
			1.04			1.63
			1.56			1.67
			1.67			2.41
			3.57			3.97
Median		1.69	0.97		3.19	1.54
34	1.21	3.36	0.53	0.55	4.10	0.74
238-244		3.52	0.70		4.63	1.10
		3.84	1.27		4.86	2.33
		6.50	1.47		10.00	2.40
			2.71			3.49
			2.86			4.11
			4.37			7.80
			7.02			8.07
Median		3.68	2.09		4.75	2.94
35	1.52	3.12	1.43	1.45	2.64	2.80
245-251		3.78	2.12		3.63	3.44
		5.21	2.48		4.97	3.93
		5.64	2.70		5.27	4.36
		6.54	3.43		6.06	4.74
		6.62	3.49		6.86	6.07
		6.83	3.70		7.97	6.37
		7.05	4.54		8.95	10.09
		7.43	4.88		9.36	10.37
		9.02			11.00	
		9.54			11.16	
		13.62			11.36	
Median		6.73	3.43		7.39	4.74
-258	1.78	3.32	1.30	1.68	4.77	2.55
		2.59	1.90		5.44	3.30
		3.07	3.00		6.93	3.84
		3.63	4.25		7.58	3.84
		4.24	4.82		7.85	4.87
		7.70	5.12		8.48	5.17
		9.00	6.30		9.03	5.88
		9.44	6.35		9.25	6.88
		11.86	7.38		9.67	7.97
		14.50	8.22		10.00	10.08
		16.40	8.58		13.14	11.4
			10.14		15.00	13.86
			14.28		15.76	17.49
					17.17	
					19.78	
					36.00	
					44.36	
Median	7.70	11.60	6.30	6.42	9.62	5.88
37	2.95	2.44	4.16	2.49	3.09	5.80
259-265		2.61	4.41		4.08	6.24
		4.43	4.58		5.49	6.37
		5.68	4.64		6.11	6.60
		6.24	6.70		7.83	7.11

Gestational age (days)	ABC (Lecithin)	DF (Lecithin)	Control (Lecithin)	ABC (L/S ratio)	DF (L/S ratio)	Control (L/S ratio)
	7.98	7.14	6.94	10.06	8.03	7.37
	9.28	10.06	7.69	10.73	9.27	8.50
	9.86	11.17	7.93	10.30	9.47	8.81
	9.90	12.30	8.67	10.76	10.47	8.94
	9.96	14.43	8.80	11.96	11.84	8.95
	10.03	16.70	9.93	13.46	12.33	9.51
	10.55	20.13	10.68	13.72	19.42	10.53
	10.86	37.78	11.60	13.75	39.77	12.57
	11.07		12.22	14.10		13.03
	12.76		12.74	15.37		14.07
	13.99		16.52	15.90		16.2
	14.57			16.01		
	15.40			17.91		
	16.76			19.54		
Mean	9.96	10.06	8.30	11.96	9.27	8.88
SD	3.33	4.05	2.13	6.17	4.93	1.78
	5.33	5.00	2.54	7.51	5.33	4.70
	9.78	5.03	4.74	13.40	5.62	5.71
	11.10	5.68	7.16	13.67	6.04	9.06
	11.48	7.58	8.40	17.08	7.58	9.78
	14.61		9.36	20.01		10.7
			13.47			11.51
			18.10			14.00
			18.22			15.48
			18.96			16.41
			24.78			18.06
Mean	10.44	5.03	9.36	13.59	5.62	10.77

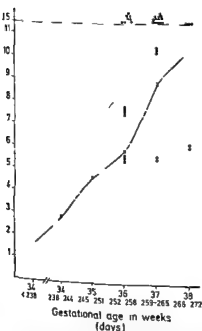


Fig 1 Amniotic fluid lecithin/sphingomyelin (L/S) ratios from 94 samples from pregnant diabetic women related to gestational age. ● One sample from a diabetic woman. — Median values for the diabetics. — Median values for 65 non-diabetic controls.

The diabetics have been divided into two groups according to White's classification. One group consists of classes A, B, C and the second group of classes D, E, F which had diabetic retinopathy and/or nephropathy. The data from these two groups have been evaluated and compared with each other and with the reference series mentioned above.

The results from the diabetic samples obtained within one week of delivery have been related to the development and severity of the RDS. These findings have been compared with a second reference group consisting of rhesus immunized and normal patients. The assessment of the respiratory function of the newborn has been as previously published (10). In addition respiratory distress requiring supplemental O_2 for less than 24 hours had been defined as transitory.

RESULTS

Fig 1 shows the calculated L/S ratios from 94 diabetic samples related to gestational age. The data for lecithin concentrations and calculated L/S ratios are listed in Table I. The median values for both parameters in the total diabetic group are higher from <34–37 weeks gestation than the median values for the reference group, whereas at 38 weeks the median values are lower than at 37 weeks and also lower than the reference group. The differ-

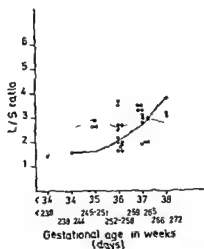


Fig 2 Amniotic fluid lecithin/sphingomyelin (L/S) ratios in 59 samples from pregnant diabetic women (Gluck's method) related to gestational age. ● One sample from a diabetic median values for the diabetics — median values for 41 non-diabetic controls

ence for the L/S ratios <34 weeks gestation is marginally significant $P=0.05$ by the Wilcoxon two-sample test but there is no significant difference between the groups at weekly intervals from 34–38 weeks gestation. However combining the data from <34–37 weeks gestation gives a difference which is highly significant $P<0.001$.

Fig 2 shows the L/S ratios in 59 randomly unselected diabetic samples analyzed by Gluck's method. 49 of these samples are included in Fig 1. The median values for the diabetics are higher from 36 weeks than the reference group. Otherwise results demonstrate the same tendency as seen in Fig 1 with generally lower L/S ratios due to the methodological difference.

In Fig 3 the patients have been divided into two groups according to White's classification A, B, C in one group and D, F in the other. The median values for classes A, B, C—39 samples—increase from 36–38 weeks gestation and are higher than the corresponding values for the reference group. The difference is not significant. We have no results for classes A, B, C prior to 36 weeks gestation. The median phospholipid concentrations in classes D, F rise steeply from <34–36 weeks gestation and are markedly higher than the corresponding values for both the reference group and classes A, B, C. At 36 weeks gestation there is a significant difference for the L/S ratios of classes D, F vs controls $P<0.05$. For the period <34–36 weeks the difference is highly significant $P<0.001$. At 37 weeks the me-

dian values for classes D, F are slightly lower than at 36 weeks and at 38 weeks they are markedly lower than both the controls and the A, B, C groups. Statistical analysis of the difference between classes D, F and A, B, C respectively vs controls at 38 weeks were considered invalid because these patients represent a selected group.

The 65 diabetic patients included in the study gave birth to 66 infants. Eleven of these developed RDS (~17%). In addition 6 infants had transient respiratory distress. Altogether 26% of the infants had some symptoms of respiratory distress but none died and only one child was considered to have a life-threatening illness.

Figs 4 and 5 present phospholipid concentrations in samples from 55 non-diabetic patients delivered within one week of delivery. In this group the clinical picture of transitory RDS has not been reported. Nine infants had RDS and all had lecithin concentrations $<4.6 \mu\text{M}/100 \text{ ml}$ and L/S ratios <1.1 . Infants with higher lecithin concentrations had normal respiratory function.

Figs 6 and 7 present the lecithin concentrations and the calculated L/S ratios in infants of diabetic mothers obtained within

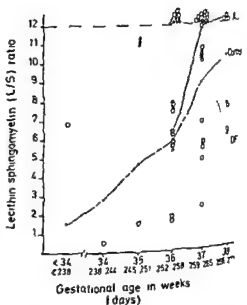


Fig 3 Amniotic fluid lecithin/sphingomyelin (L/S) ratios in 93 samples from pregnant diabetic women (one patient unclassified) related to gestational age. ○ One sample from a diabetic classes A, B, C ● one sample from a diabetic classes D, F — median values for classes A, B, C — median values for classes D, F — median values for 41 non-diabetic controls

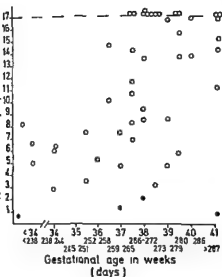


Fig 4 Amniotic fluid lecithin concentrations obtained within one week of delivery from 55 non-diabetic patients related to gestational age ○ No RDS ● RDS

k of delivery For the remaining 7 infants in group the interval between amniocentesis and delivery was more than one week. The majority collected at caesarean section. We have previously found RDS constantly to be associated with low phospholipid concentrations. In this dia-

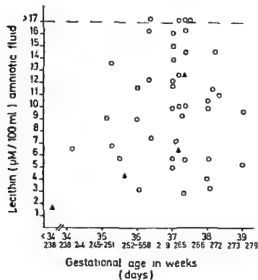


Fig 6 Amniotic fluid lecithin concentrations obtained within one week of delivery from 58 diabetic patients related to gestational age ○ No RDS ▲ transitory RDS ● RDS

betic series RDS occurred even with very high phospholipid concentrations. Ten patients with lecithin concentrations $>4.6 \mu\text{M}/100 \text{ ml}$ and 7 patients with lecithin concentrations $<4.6 \mu\text{M}/100 \text{ ml}$ developed RDS. Of these there were 12 with a L/S ratio >5.0 and 5 with L/S ratio <5.0 .

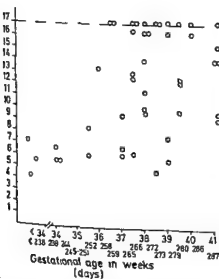


Fig 5 Amniotic fluid lecithin/sphingomyelin (L/S) ratios obtained within one week of delivery from 55 non-diabetic patients related to gestational age ○ No RDS ● RDS

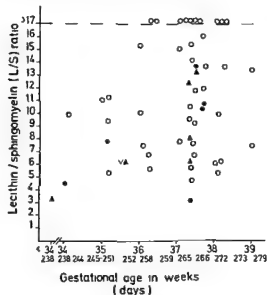


Fig 7 Amniotic fluid lecithin/sphingomyelin (L/S) ratios obtained within one week of delivery from 58 diabetic patients related to gestational age ○ No RDS ▲ transitory RDS ● RDS

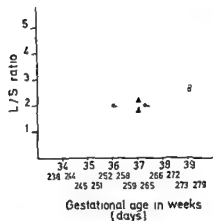


Fig 8 Amniotic fluid lecithin/sphingomyelin (L/S) ratios obtained within one week of delivery from 33 diabetic patients (Gluck's method) related to gestational age. ○ No RDS, ▲ transitory RDS, ● RDS

Fig 8 illustrates the L/S ratios in 33 of the diabetics analysed by Gluck's method. These were unselected samples obtained within one week of delivery. There were 7 cases of RDS and 2 additional cases of transitory RDS. Eight of these had L/S ratio ≥ 2.0 . In neonates from non-diabetics we have hitherto not seen RDS with a ratio ≥ 2.0 by this method.

The relationship between the mode of delivery and respiratory function is difficult to assess since the majority (~80%) were delivered by caesarean section. The proportion of RDS was approximately the same with either route of delivery.

Table II shows the Apgar scores for 61 neonates. There is a preponderance of low Apgar scores in the group with RDS. The Fisher-Irwin test demonstrates a highly significant correlation between asphyxia at birth and subsequent clinical outcome, $P=0.01$ with the two-sided test.

In a preliminary study of the maternal urinary oestrol values there seemed to be no obvious correlation either to phospholipid levels or respiratory

function in the newborn. We were also unable to find any relationship between birthweight, phospholipid concentrations at any given gestational age. The incidence of RDS was the same in both sexes.

Nearly all diabetics in this study were well-controlled, especially during the last trimester. The importance of poor maternal control with respect to RDS is therefore impossible to assess in this trial.

There is a slight overrepresentation of the offspring of diabetics in classes D and F. This difference is not significant.

DISCUSSION

In the present study there is no general evidence of delayed maturation of the pulmonary surfactant system in diabetic pregnancies. On the contrary, from <34–37 weeks gestation there are significantly higher phospholipid concentrations in the samples from the diabetic patients than in the reference group (Figs 1 and 2). The results at 38 weeks are inconclusive. It is partly a selected group of patients where delivery was postponed because of low phospholipid concentrations in earlier samples. Whitfield et al. (18) found that the normal terminal rise in the L/S ratio did not occur in approximately one-third of the diabetic patients. Evidently there is a delay in the normal terminal rise of the L/S ratio in some of our patients, but we have observed this in other groups of patients and even in pregnancies considered to be normal. Whitfield et al. also noted a steep fall in the L/S ratio in three patients. A similar fall was observed in two of our patients at this stage of gestation. The extent to which low values in classes D and F are due to a fall or a delayed maturation will be examined in further studies.

It is seen in Fig 3 that nearly all samples obtained before 36 weeks gestation are in White groups D and E. Our findings of a highly significant difference between groups D and F and the reference series strongly support Gluck and Kulovich's theory of accelerated maturation of the surfactant system in these groups. Their suggestion of retardation in classes A, B, and C is, however, not in accordance with our results. For these groups we find a steady increase in the phospholipid concentrations parallel to the reference group. The median values are higher than in the reference group, but the difference is not

Table II Relationship between Apgar scores and subsequent RDS

Apgar scores	No. of patients	RDS	No RDS
3–7	11	7	4
8–10	50	10	40

ant This is in agreement with other reports

At the present time there is general acceptance of the use of phospholipid concentrations in amniotic fluid to indicate minimal risk of RDS in the newborn. With our method mature lecithin concentration $>4.6 \mu\text{M}/100 \text{ ml}$ and L/S >5.0 . With this method delivery is considered safe with an L/S ≥ 2.0 . In our reference group (Figs 4 and 5) there is no case of RDS among those with mature

In the diabetic group, however, there are several cases of RDS with mature concentrations (Figs 6, 7 and 8). The occasional occurrence of RDS in infants of diabetic mothers despite mature L/S has been observed by several authors and has been regarded as exceptional but the frequency with which it occurred in our group makes it clear that mature values are no guarantee against respiratory distress.

Since much of our measurements have been made on amniotic fluid it is interesting to note that Johnson et al. (1) found normal lecithin content and surface tension properties in the lungs of three infants of diabetic mothers who died of hyaline membrane disease (HMD). In their study this was not unusual and they suggested that the HMD seen in infants of diabetic mothers was of a unique kind. We also found evidence that these infants could be exceptionally well endowed with surfactant.

At present we have no adequate explanation for the occasional occurrence in the presence of ample surfactant. An important factor is asphyxia at birth as demonstrated by our finding of a highly significant relationship between low Apgar score and the subsequent development of RDS. This confirms the findings in other studies (3, 4, 7, 13). On the other hand, some infants with mature phospholipid concentrations and no asphyxia at birth developed RDS. In our series there were no deaths from RDS. It should be pointed out that the majority were mild to be mildly affected only. It is our experience that the condition of the newborn has improved considerably since stricter control of the maternal diabetes was introduced. Both mortality and morbidity from RDS have been reduced. Confronted with the paradox of RDS developing despite adequate phospholipid concentrations it is possible that even stricter control of the maternal diabetes to improve metabolic and circulatory factors in the newborn of importance for respiratory function.

ACKNOWLEDGEMENTS

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SECONDARY AMENORRHOEA AND ORAL CONTRACEPTIVES

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Eighty seven cases of secondary amenorrhoea of more than 6 months duration developing after treatment with oral contraceptives (group I) were compared with 27 cases of secondary amenorrhoea not preceded by treatment with combined tablets (group II). The two series were collected during the same period. The average age of the patients was 4 years higher in group I than in group II.

Oligomenorrhoea had previously occurred in 46% of group I and in 46% of group II. Pronounced premenstrual factors such as psychogenic trauma and stress or without considerable change in body weight were present in 26% in group I and 56% in group II. The percentage of increased urinary output of 17 keto steroids, of ketogenic steroids and of hirsutism was slightly higher in group I. The percentage of eosinophilic cells in vaginal smears was low in 70% in group I as compared with 40% in group II. Spontaneous return of pituitary-ovarian function occurred in 40% in both groups. Patients recovering spontaneously in group I presented a maximum of the first few months followed by a steady and uniform decline. Spontaneous recovery in group II was more or less independent of time. It seems reasonable to believe that oral contraceptives did promote or contribute to the development of secondary amenorrhoea in 150% representing cases with various predisposing factors. A causal relation between oral contraceptives and secondary amenorrhoea was indicated in the remaining cases because of perfectly normal ovarian function before treatment and absence of predisposing factors.

Treatment with tablets containing oestrogens and progestagens (oral contraceptives) brings about within a few months a considerable suppression of pituitary gonadotrophic hormones and of ovarian endocrine function. When the treatment is discontinued endocrine activity is re-established immediately in most cases. Some delay is seen in a certain percentage and secondary amenorrhoea develops in a few cases. The first case of secondary amenorrhoea following treatment with oral contraceptives was published in 1966 (13). Since then a

large number of reports have appeared in which the relation between oral contraceptives and secondary amenorrhoea is discussed. The fact that this relation is still obscure prompted us to approach the problem in a comparative study of two groups of patients with secondary amenorrhoea: the first group having used oral contraceptives while the second had not received any preceding hormonal treatment.

MATERIAL AND METHODS

The series studied included 87 cases with secondary amenorrhoea starting immediately following treatment with oestrogen gestagen tablets (group I) and 27 cases with secondary amenorrhoea not preceded by hormonal treatment (group II). All patients were referred to The State Maternity Hospital, University of Aarhus. It seems reasonable to believe that most, if not all, cases occurring in the surrounding area—a city with a population of about 250 000 inhabitants—were seen. In addition the series included a number of cases from more distant areas referred to us by gynaecological departments, consultants and general practitioners.

Secondary amenorrhoea was defined as amenorrhoea of more than six months duration including all cases irrespective of the menstrual pattern preceding the amenorrhoea. Cases due to obvious organic disease were not included in any of the two groups which were collected from January 1966 to July 1972.

On admission to the hospital all the patients were subjected to an endocrine examination including determination of urinary output of 17 keto steroids, 17 ketogenic steroids and total gonadotrophins by the routine methods applied by the Danish State Serum Institute. Abnormal results were confirmed by repeated examinations. In addition basal metabolic rate, PBI and triiodo thyronine were measured. The percentage of eosinophilic cells in vaginal scrapings was assessed in three examinations. Radiological examination of the sella turcica and testing of the visual fields were carried out. Biopsy of the endometrium and chromosome studies were not performed routinely.

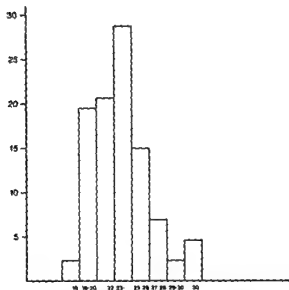


Fig 1a The distribution of patients in group I on the basis of age at the onset of amenorrhoea

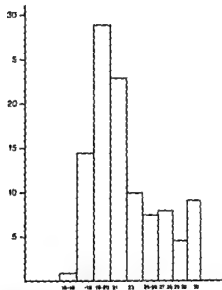


Fig 1b The distribution of patients in group II on the basis of age at the onset of amenorrhoea

RESULTS

A few facts concerning oestrogen gestagen treatment will be considered before a detailed description of the two groups is given.

The indication for treatment with combined tablets was mainly a demand for contraception (69%) or irregular usually infrequent periods (28%). In two cases treatment was instituted because of dysmenorrhoea. The composition of our series is fairly similar to that reported by other authors (e.g. 6, 9). During treatment perfectly regular withdrawal bleeding occurred in 92%. The treatment was discontinued because the patients wanted to become pregnant (23%) because of side effects (22%) and because it was supposed to

be reasonable to stop for a while (19.5%). No definite reason was stated in about one third of the cases.

The age distribution of the two groups (Fig. 1) disclosed a significant difference ($p=0.01$), the average being 4 years higher in group I than in group II. The body weight of the patients is illustrated in Fig. 2, which is based on weights and heights according to Danish standards (life insurance tables). The distribution is fairly uniform and both groups include a considerable number of cases below the ideal.

No conspicuous difference was demonstrated between the age at the menarche (Fig. 3) in the two groups although the average was slightly higher

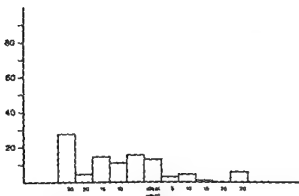


Fig 2a The distribution of patients in group I on the basis of weight and height

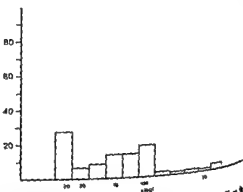


Fig 2b The distribution of patients in group II on the basis of weight and height

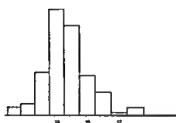


Fig 3a The distribution of patients in group I on the basis of age at the menarche

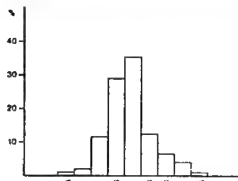


Fig 3b The distribution of patients in group II on the basis of the age at the menarche

The menstrual pattern preceding secondary amenorrhoea was compared oligomenorrhoea defined as vaginal bleeding occurring at intervals of 45 days or more. The percentage of normal periods before the use of oral contraceptives was 55 as compared with 50 in group I and 30% in group II. The difference in the occurrence of oligomenorrhoea is significant ($p=0.05$). The relation between psychogenic factors and secondary amenorrhoea has been demonstrated by a number of investigators (for references see 6, 7). In the present series all cases with psychogenic amenorrhoea were recorded provided a close chronological relationship was demonstrated between psychogenic trauma or massive stress with amenorrhoea, a major change in body weight, for example divorce, a temporary stay abroad or a change of residence. Although the record includes subjective factors it should be possible to avoid bias when both groups are compared according to the same criteria and by the same investigator. A predisposition of this nature was present in 76% of the patients in group I and in 50% in group II. The difference is significant ($p<0.05$). Amenorrhoea defined as a complaint of spontaneous milk-like secretion from one or both breasts occurred in one case in group I and in four cases in group II. Figures presented in the literature are within wide limits. This variation may be due to methodological differences (5, 10, 14) although it is reasonable to assume that differences depend on a search for and inclusion of cases disclosed by manual expression of the breasts. The incidence as assessed by this approach

is considerable in parous women and independent of treatment with contraceptive drugs (14).

A detailed discussion of the treatment and prognosis in the two groups is not appropriate in this context because the approach to treatment was not absolutely identical. Patients with secondary amenorrhoea occurring after the use of oestrogen-gestagen tablets were followed without any treatment being given apart from those complaining of sterility whereas the attitude towards treatment was more liberal in cases belonging to group II. The incidence of spontaneous recovery of pituitary-ovarian function was 40% in both groups representing minimum values based on follow-up periods of varying lengths and calculated on the total number in each group without respect to cases dropping out of the follow-up study. The intervals between the onset of secondary amenorrhoea and the first bleeding indicating recovery are presented in Fig 4 which shows a substantial difference between the two groups.

Reports on the return of ovarian function after secondary amenorrhoea are contradictory on several points. The length of the period justifying the use of the term varies in individual papers. Particular attention is usually not paid to cases due to organic disease. Many of the series published comprise only a limited number of cases and the follow-up periods are often rather short. Very good results were obtained in a Swedish series (8) with spontaneous remission in 69% of 178 cases of secondary amenorrhoea developing after hormonal treatment with follow-up periods up to 39 months.

Finally it should be mentioned that in our series the percentage of patients in whom spontaneous return of ovarian function occurred after treatment

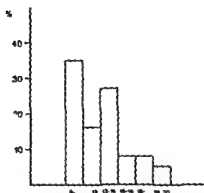


Fig 4a The distribution of patients in group I on the basis of the first bleeding indicating re-establishment of ovarian function

with oral contraceptives was roughly alike in patients who had a perfectly normal menstrual pattern and in those with oligomenorrhoea before treatment was started. However the tendency to recovery in group I depended to a certain extent on the number of years which had elapsed between the menarche and the start of treatment with combined tablets: in women who started 5 years or more after the menarche ovarian function was re-established in 32% as compared with 42% in those who started less than 5 years after the menarche.

DISCUSSION

The relation between treatment with oral contraceptives and the development of secondary amenorrhoea has been widely discussed. The difference of opinion is reflected in the terms post-contraceptive amenorrhoea (9) and the oversuppression syndrome (1). On the other hand many authors question the relation or completely reject any sort of relationship, calling attention to the occurrence of functional amenorrhoea in the population although the incidence is so far questionable (12-15). Attention has also been directed to psychogenic factors and to the uniformity of all cases of secondary amenorrhoea as far as clinical, prognostic and therapeutic aspects are concerned although systematic comparisons of cases with and without preceding treatment with oral contraceptives do not seem to be available.

Within a few months treatment with oral contraceptives produces a considerable depression of pituitary and ovarian function, presumably due to inhibition of the release of the hypothalamic releas-

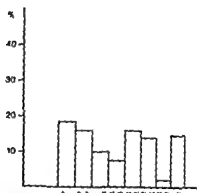


Fig 4b The distribution of patients in group II on the basis of the first bleeding indicating re-establishment of ovarian function

ing factor. When treatment is discontinued, ovarian function is promptly re-established in about 75% of cases, to a certain extent depending on the type of treatment administered and the composition of the tablets. The great majority of the remaining patients present delayed periods, ovulatory or anovulatory cycles, quite often with some irregularity. A few patients experience amenorrhoea of more than 6 months duration. The frequencies of secondary amenorrhoea observed in series comprising 1000 to 20000 cases vary from 2-3/1000 (8) to 7/1000 (10) although higher figures have been reported in small series, for example 2.7% (2). In conclusion it seems reasonable to consider the clinical picture of secondary amenorrhoea as a spectrum from immediate onset on the one hand to long standing amenorrhoea on the other.

The incidence of secondary amenorrhoea in the total population has not been established. Investigations related to this problem have been based on selected samples of the population, almost all of whom had been exposed to stress of a varying nature (3-4). Accordingly it is not possible to decide whether secondary amenorrhoea following discontinuation of contraceptive treatment occurs with an incidence differing from that in fertile women in general. It is obvious that cases of secondary amenorrhoea following the use of oral contraceptives will involve an increasing percentage of the total number of women in the course of time. The series presented here do not allow a comparison between the incidence of secondary amenorrhoea year by year and the increase in the use of oral contraceptives. About 25% of the total number occurred after treatment with oestrogen-gestagen tablets, but this percentage represents a minimum value because the corresponding

was started in 1966 the very year when oral contraceptives were introduced in this country. It must be stressed that the majority of patients came from an area with a considerable number of institutes of higher education which implies that the basic series contained a comparatively high percentage of young and very young women with secondary amenorrhoea which is not due to organic disease. It is generally monosymptomatic. Thus it does not seem reasonable to question the influence of oral contraceptives as a predisposing factor just because the clinical appearance of secondary amenorrhoea is similar to the control group. A detailed analysis of the findings in the two series disclosed some minor and a few major differences. The average age in group I was four years younger than in group II. Predisposition such as psychogenic trauma, stress and considerable increase in body weight occurred in 26% in group I compared with 56% in group II. Furthermore, group II included more cases with an increased urinary output of 17 keto steroids and 17 ketogenic steroids and rather more hirsute patients. In addition, the percentage of eosinophilic cells in the vaginal smears was 46 in group I and 20 in group II. In comparison of the case records disclosed that the average age at the menarche was slightly higher in group II. Oligomenorrhoea during the year preceding treatment with oral contraceptives occurred in 40% as compared with 46% during the year preceding amenorrhoea in patients in group II. The reasons already specified for the two groups were not compared as regards the course of the disease. In general, the treatment employed or the duration of treatment. Making a reservation for the reasons mentioned, it must be stressed that 40% of the patients experienced spontaneous recovery after recovering after treatment with oral contraceptives presented a maximum during the first 6 months followed by a steady and rather uniform decline. As far as group II is concerned, spontaneous recovery was more or less independent of time.

Attention to the differences mentioned between the two groups I and II, it seems reasonable to assume that oral contraceptives promote or contribute to the development of secondary amenorrhoea in the same predisposing factors as in patients with secondary amenorrhoea which has not been preceded by hormonal treatment of this nature may be enough to explain the

development of secondary amenorrhoea in about 50% of cases occurring after treatment with oral contraceptives. As far as the other 50% are concerned, the presence of perfectly normal pituitary-ovarian function before treatment, the absence of predisposing factors and the close time relation between discontinuation of treatment and secondary amenorrhoea are suggestive of a causal relationship. Secondary amenorrhoea may be regarded as an extreme type of reaction in the pattern seen during the period after stopping oral contraceptives. This view is supported by a comparison between the fairly slight suppression of pituitary-ovarian function seen after sequential treatment, the moderate suppression produced by combined tablets and the pronounced effect of treatment with gestagens administered by injection every third month. The increasing degree of suppression is reflected by the endometrial reaction (11) and by the tendency to develop secondary amenorrhoea.

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PLASMA HORMONE CONCENTRATIONS DURING THE MENSTRUAL CYCLE OF NORMAL CHINESE WOMEN

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Abstract The concentrations of progesterone, estrogens, luteal phase serum-inhibiting hormone and follicle stimulating hormone were measured in samples of plasma taken frequently during 10 menstrual cycles in fourteen Chinese women. The pattern of changes in the concentrations of the hormones during the menstrual cycles was characterized by changes which were comparable to those previously reported in Caucasian subjects.

Hormone profiles in normal cycles of Caucasian women have been well documented (1, 4, 6, 7, 9, 11). However, there has only recently been a report of the concentrations of hormones during the menstrual cycle of an Asian population (8). As a prelude to establishing an ovulation induction programme, we considered that it was essential to determine the concentrations of hormones in normal menstrual cycles of women native to Singapore. In the present study, progesterone, estrogens, luteal phase serum-inhibiting hormone (LH) and follicle stimulating hormone (FSH) were measured in fourteen normal Chinese subjects.

PATIENTS AND METHODS

Subjects

Fourteen normal healthy Chinese women with regular menstrual cycles were studied. All subjects were nulliparous. Their ages ranged from 21 to 32 (mean 24.6) years. Blood samples were collected at the same time every day from day 70 of the cycle, before and after this period. Sampling was performed every other day. The blood was collected from the syringe to a lithium heparin tube. The tube was centrifuged and the plasma removed. Aliquots of plasma were removed and stored at -20°C until the assay was completed.

Progesterone assay

Progesterone was assayed in duplicate using a specific radioimmunoassay based on a previous report (2). The

specific antiserum against progesterone used in the present study was generated in sheep by the injection of 11-hydroxy progesterone hemisuccinate conjugated to BSA. Plasma samples (0.5 ml) were extracted in duplicate with petroleum ether (30-40). The extracts were dried and dissolved in tris buffer (pH 7.8) containing 0.1% gelatin (1.5 ml). Duplicate aliquots (0.5 ml) of the extract in buffer were removed for assay. Antiserum at a concentration of 1:1000 (0.1 ml) and tritiated progesterone (0.1 ml) containing approximately 10000 dpm were mixed with the sample. After an incubation period of 18-24 hours at $0-4^{\circ}\text{C}$, dextran coated charcoal was added to adsorb the free steroid. The tubes were centrifuged for ten minutes in the cold and an aliquot of the supernatant (0.5 ml) was placed in a liquid scintillation vial containing 10 ml toluene based scintillation fluid with Triton X 100. The radioactivity was determined in a Nuclear Chicago Mark II Liquid Scintillation Counter. The progesterone concentration was calculated from standard curves and were corrected for losses during extraction. Control samples taken through the procedure revealed that the inter- and intra-assay coefficient of variation was less than 15%. Chromatographic separation of progesterone was not performed as the antiserum was shown to be highly specific for progesterone.

Assay of total estrogens

An aliquot of plasma was extracted with freshly distilled diethyl ether. The total unconjugated estrogens in the extract were measured by a radioimmunoassay based on the method of Speroff and co-workers (10) although column chromatographic separation of the individual estrogens was not performed. The antiserum was used at a concentration of 1:18000 and approximately 20000 dpm $6,7\text{-}^3\text{H}\text{-}17\beta\text{-estradiol}$ (SA 56 Ci/mM, The Radiochemical Centre) was added to the assay tube. After a 18-24 h incubation at $0-4^{\circ}\text{C}$, free and bound hormone were separated by dextran coated charcoal. The radioactivity in an aliquot of the supernatant (0.5 ml) was measured as described in the assay of progesterone.

The antiserum reacts with estrone, estradiol and estrinol in the following ratios: 100:30:3 respectively (10). The results were expressed as estradiol equivalents per ml of plasma.

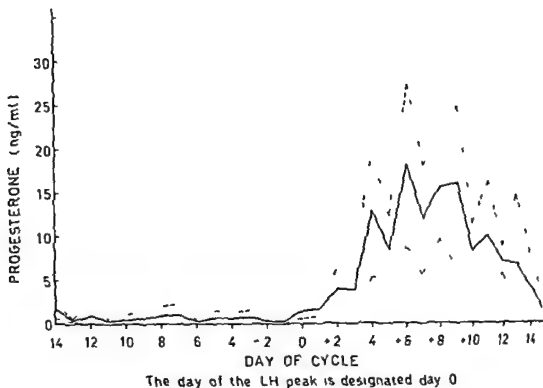


Fig 1 The mean and one standard deviation of plasma progesterone concentration during menstrual cycles in 14 Chinese subjects

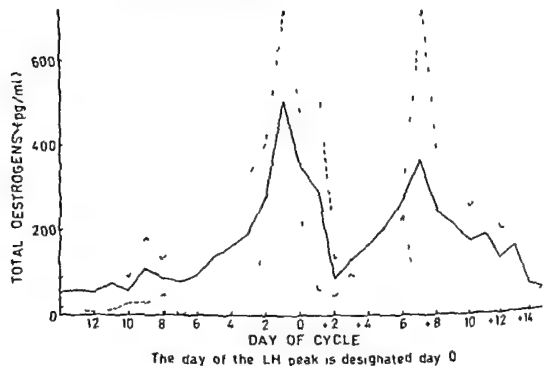
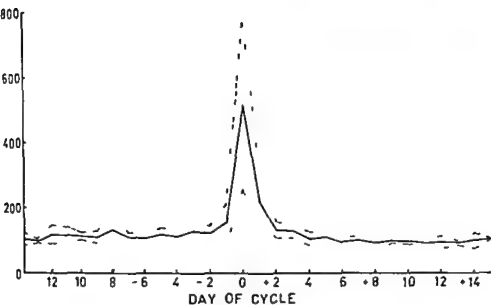
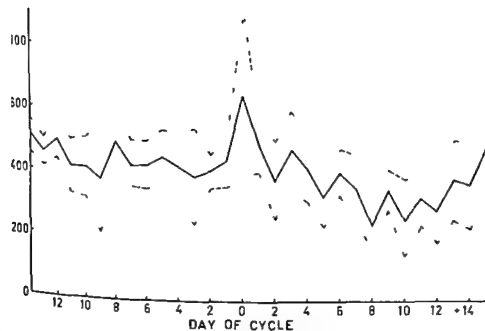


Fig 2 The mean and one standard deviation of plasma total oestrogen concentration during menstrual cycles in 14 Chinese subjects



The day of the LH peak is designated day 0

The mean and one standard deviation of plasma luteinizing hormone concentration during menstrual cycles in 14 Chinese subjects



The day of the LH peak is designated day 0

The mean and one standard deviation of plasma follicle-stimulating hormone concentration during menstrual cycles in 14 Chinese subjects

Assay of luteinizing hormone (LH)

LH was estimated by a double antibody, disequilibrium radioimmunoassay similar to that described by Midgley and others (5) using reagents supplied by the National Pituitary Agency, National Institutes of Health, Bethesda, USA. Iodination of high grade LH (LER 960 (2.5 µg) was performed using Na¹²⁵I by the method of Greenwood, Hunter and Glover (3). Specific activities of approximately 100 µCi/µg were obtained. Rabbit anti-LH serum (Batch no. 1) was used at a 1:8000 dilution and this bound approximately 30% of added tracer. The plasma sample (0.1 ml) was incubated at 0–4 °C with the antibody for one day, then the ¹²⁵I hormone was added and incubation continued for a further three days. The second antibody (sheep anti-rabbit gamma globulin) was added and incubated for another three days in the cold. After centrifugation in the cold, the supernatant was discarded and the precipitate counted in a Nuclear Chicago Gamma Spectrometer. The reference preparation employed was LER 907 which has recently been assigned the following immunoreactivity: 1 mg LER 907 = 60 IU of LH and 20 IU FSH 2nd IRP HMG.

Assay of follicle stimulating hormone (FSH)

FSH was measured by a radioimmunoassay similar to that for LH. The reagents were also supplied by the National Pituitary Agency, National Institutes of Health, Bethesda, USA. The rabbit anti-FSH serum (Batch no. 3) was used at a 1:4000 dilution which was capable of binding approximately 20% of added tracer (¹²⁵I LER 1366 at specific activities of approximately 100 µCi/µg).

RESULTS

All fourteen subjects menstruated regularly and had cycle lengths between 26 and 32 days (mean 29.2). The lengths of the follicular and luteal phases as determined from the peak of LH activity were 10 ± 0.6 (range 12–20) and 14.2 ± 0.3 (range 12–16) days respectively.

Using the mid-cycle surge of LH as a reference, composite graphs of progesterone, total estrogens, LH and FSH were constructed and are illustrated in Fig. 1–4.

Progesterone levels during the follicular phase were 0.63 ± 0.10 ng/ml and the level began to rise on the day of the LH peak to a maximum of 17.9 ± 4.2 ng/ml 6 days after the surge of LH.

The concentration of estrogens was usually highest on the day preceding the LH peak although in a few cases it coincided with the LH peak. The peak level of total estrogen was 508.8 pg/ml. A secondary peak was observed which paralleled the profile of progesterone, having a peak 7 days after the LH surge of 364.4 pg/ml.

The mean mid-cycle peak of LH was 512.7 ± 132.3 ng LER 907/ml and the levels during

the luteal phase were 98.7 ± 12.0 ng LER 907/ml which is slightly lower than during the follicular phase (112.7 ± 11.0 ng LER 907/ml). The pattern of FSH was not so well defined as LH but it did rise on the same day as LH (662.1 ± 81.7 ng LER 907/ml).

DISCUSSION

The hormone profiles observed during the present investigation were similar to those in Caucasian women (1, 4, 6, 7, 9, 11, 12) and Thai women (8).

Progesterone levels were similar to those reported in Caucasian women although lower than those reported in Thai women. However, the present study used a different assay procedure (competitive protein binding) and the mean values were affected by the unusually high concentrations of progesterone measured in two of the six subjects studied.

The concentrations of total estrogens are comparable to those in Caucasian and Thai women.

The levels of LH during all phases of the menstrual cycle are slightly higher than those reported in Thai women (8) using the same standard preparation. Other investigators have used different reference preparations and it is therefore difficult to give direct comparisons. However, calculations of LH obtained in Caucasian women in terms of LER 907 reveal similar levels to those reported in the present study. The LH concentration is slightly lower than that in the follicular phase due to the inhibitory feedback response of the steroids during the luteal phase.

It is also difficult to make direct comparisons of our FSH data with similar data from other studies due to the use of a variety of standard preparations. However, the levels and certainly the profiles of FSH in plasma appear to be similar. There was much larger inter-subject variation in the concentration of FSH compared with LH. The concentration of FSH during the early follicular phase was high but it did drop before the mid-cycle surge. The steroid produced during the luteal phase caused a reduction of the FSH level in the blood by the feedback response on the hypothalamus, but a rise of FSH was observed prior to menstruation.

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E COLI GROWTH INHIBITION BY AMNIOTIC FLUID

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Amniotic fluid samples were collected aseptically from 29 normal pregnancies between the 38th and 41st week of gestation and checked for sterility in the laboratory in order to investigate in vitro the effect of the fluid on the growth of *E. coli*. Brain Heart Infusion (BHI) and Ringer solution were used as controls. *E. coli* strains were inoculated in amniotic fluid, BHI, Ringer solution and amniotic fluid plus BHI and incubated at 37°C for 48 hours. At 0, 1, 3, 6, 12, 24, 36 and 48 hours incubation, surface viable counts were performed to estimate the number of *E. coli* viable cells. The growth of *E. coli* in BHI started during the 1st hour after inoculation and continued over 48 hours. In Ringer solution the growth curve was almost identical to that of BHI. In amniotic fluid the growth of *E. coli* began from the 1st hour of inoculation but the growth curve was much lower and became static in 74 hours and a permanent inhibition was observed thereafter. The addition of a small amount of BHI in amniotic fluid enhanced the growth of *E. coli*. The growth curve was lower in comparison to the results of the two controls studied. In conclusion, after 48 hours of inoculation in amniotic fluid, 76% of the 29 cases showed bacteriostatic or bactericidal activity of the liquor on the growth of *E. coli*.

Antibacterial activity in amniotic fluid was reported for the first time by Cattaneo (2) who suggested the presence of lysozyme, while Gusdon (6) found a substance bactericidal for *Bacillus subtilis* which did not appear to be lysozyme. In approximately 50% of the amniotic fluids tested. Contrary to the above authors, Walsh et al (11) and Sarkany & Sylva (10) reported that human amniotic fluid does not exhibit antibacterial activity and generally acts as a good culture medium for bacteria. More recently Galask & Snyder (4, 5) have shown that amniotic fluid is inhibitory for a number of Gram positive and Gram negative bacteria and have attempted to identify the antibacterial factors in it. Similar results were reported by Bergman et al (1)

who considered various antibacterial factors to exist in amniotic fluid acting alone or in combination.

Inhibitory effects on the growth of *E. coli* in amniotic fluid were reported by Galask & Snyder (4), Florman & Teubner (3) and Kitzmiller et al (7) which seemed to reflect the absence of nutrients in the liquor. Furthermore, Larsen et al (8, 9) have demonstrated by measuring bacterial growth spectrophotometrically that amniotic fluid fails to support the growth of *E. coli* but this inhibitory effect is lost when amniotic fluid is diluted in water or in a chemically defined medium (DDM).

The aim of the present study is to investigate the effect of amniotic fluid on the growth of *E. coli* in order to determine any bacteriostatic or bactericidal activity of the fluid.

MATERIAL AND METHODS

Amniotic fluid samples were collected aseptically during cesarean section or by abdominal amniocentesis from 29 normal pregnancies between the 38th and 41st week of gestation. All the above cases were uncomplicated pregnancies and had not received chemotherapy.

Samples of amniotic fluid contaminated by blood or meconium were discarded from this study. At the beginning of the collection the first 10 ml of the liquor were discarded to avoid any mixture with traces of blood while the remainder was centrifuged at 3000 r.p.m. for 15 min and the supernatant was kept in the deep freeze at -30°C. The sediment was then checked for sterility.

For this purpose the sediment was cultured: (a) Aerobically on blood agar, McConkey agar (BBL), MRS agar (Oxoid) and on Sabouraud maltose agar (BBL); (b) Anaerobically on blood agar and in thioglycollate broth; (c) for Mycoplasmas on E. Agar and in urea broth. The supernatant was then used if it was sterile and free from blood or meconium.

Testing of inhibitory activity. Inhibitory activity of amniotic fluid was tested against *Escherichia coli*

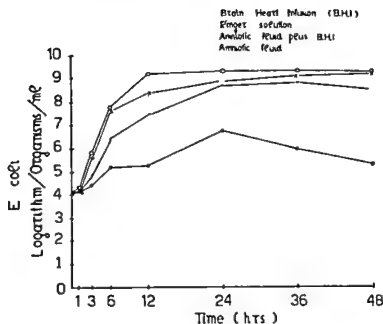


Fig. 1. Growth curves of *E. coli* in (a) amniotic fluid, (b) amniotic fluid plus BHI, (c) Brain Heart Infusion (BHI), and (d) Ringer solution (control).

NCTC 10418. Before use *E. coli* was cultured on nutrient agar and incubated at 37°C for 18 hours. Next day one loopful (by a 4 mm diameter wire loop) of *E. coli* was inoculated in 5 ml Brain Heart Infusion (BHI) and incubated at 37°C for another 18 hours. After incubation the broth-culture was diluted to 10^{-4} in BHI. From this final dilution (containing $10^5 \times 10^2$ organisms per ml) 0.1 ml was added to each of the following media:

1. To 2 ml of amniotic fluid
2. To 2 ml of amniotic fluid enriched by BHI 10%
3. To 2 ml of BHI
4. To 2 ml of Ringer solution

The last 2 tubes were controls. No. 3 checking *E. coli* growth in a favourable medium without inhibitors and No. 4 showing *E. coli* viability in the absence of any organic nutrient. Both amniotic fluids and controls were incubated at 37°C for 48 hours. At 0, 1, 3, 6, 12, 24, 36 and 48 hours of incubation surface viable counts were performed for estimating the number of *E. coli* viable cells (Miles & Mizra's method, 1938). Ringer solution was used as diluent and dilutions were made up to 10^{-4} .

RESULTS

The growth curves of *E. coli* in Brain Heart Infusion (BHI), Ringer solution, amniotic fluid plus BHI and amniotic fluid neat over 48 hours measured at 1, 3, 6, 12, 24, 36 and 48 hours by plate counting are shown in Fig. 1.

The mean values of viable counts are given in 29 experiments for amniotic fluid, in 15 for BHI, in 15 for Ringer solution and in 14 experiments for amniotic fluid plus BHI. The growth of *E. coli* in BHI started during the first hour after inocula-

tion and was continued over 48 hours. In Ringer solution the mean growth curve was almost identical to that of the other control (BHI).

In amniotic fluid the growth of *E. coli* began after the 1st hour of inoculation but the growth was much lower, became static in 24 hours and permanent inhibition was observed thereafter. Finally the addition of a small amount of BHI to the amniotic fluid enhanced the growth of *E. coli* but the growth curve was lower in comparison with the curves of the two controls studied.

Furthermore the individual growth rates of *E. coli* cells in amniotic fluid (29 cases) are listed in Table 1. As it is shown from this table bacteriostatic activity of amniotic fluid on the growth of *E. coli* was observed in two of the cases at 6 hours, six at 12 hours, six at 24 hours, eight at 36 hours and seven at 48 hours respectively.

Bactericidal activity of the fluid occurred at 36 hours in seven cases, at 36 hours in thirteen cases and at 48 hours in nineteen cases.

In conclusion, after 48 hours of incubation, the 29 cases showed a bacteriostatic or bactericidal activity of the amniotic fluid on the growth of *E. coli*.

DISCUSSION

Walsh et al. (11) and Sarkany & Gaylord (12) reported that the total viable counts of *E. coli* after 24 hours of incubation in amniotic fluid were

Table 1. Individual growth rates of living *E. coli* cells inoculated in amniotic fluid (1 ml) for 48 hours

bacteriostatic activity * bactericidal activity

Hours of inoculation								
0	1	3	6	12	24	36	48	
117 × 10 ³	708 × 10 ³	1 600 × 10 ³	5 200 × 10 ³	4 000 × 10 ³	640 × 10 ³	170 × 10 ³	8 × 10 ³ ▲	
136 × 10 ³	188 × 10 ³	600 × 10 ³	1 400 × 10 ³	1 520 × 10 ³	560 × 10 ³	160 × 10 ³	100 × 10 ³ ▲	
137 × 10 ³	240 × 10 ³	840 × 10 ³	3 200 × 10 ³	2 400 × 10 ³	240 × 10 ³	0	0	
157 × 10 ³	228 × 10 ³	520 × 10 ³	1 280 × 10 ³	480 × 10 ³	0	0	0	
136 × 10 ³	140 × 10 ³	400 × 10 ³	1 280 × 10 ³	3 600 × 10 ³	7 200 × 10 ³	170 × 10 ³ ▲	0	
28 × 10 ³	37 × 10 ³	160 × 10 ³	112 × 10 ³	56 × 10 ³	0	0	0	
20 × 10 ³	70 × 10 ³	30 × 10 ³	72 × 10 ³	42 × 10 ³	0	0*	0	
47 × 10 ³	68 × 10 ³	160 × 10 ³	440 × 10 ³	4 000 × 10 ³	640 000 × 10 ³	184 000 × 10 ³	160 × 10 ³	
74 × 10 ³	37 × 10 ³	88 × 10 ³	200 × 10 ³	400 × 10 ³	40 × 10 ³	20 × 10 ³ ▲	0	
128 × 10 ³	152 × 10 ³	840 × 10 ³	6 800 × 10 ³	3 600 × 10 ³	1 040 × 10 ³	80 × 10 ³ ▲	0	
1 4 × 10 ³	180 × 10 ³	770 × 10 ³	3 200 × 10 ³	600 × 10 ³	40 × 10 ³ ▲	0	0	
140 × 10 ³	256 × 10 ³	144 × 10 ³	1 120 × 10 ³	570 × 10 ³	40 × 10 ³ ▲	0	0*	
116 × 10 ³	100 × 10 ³	960 × 10 ³	2 800 × 10 ³	4 000 × 10 ³	680 000 × 10 ³	2 400 × 10 ³	77 × 10 ³ ▲	
100 × 10 ³	140 × 10 ³	280 × 10 ³	600 × 10 ³	480 × 10 ³	88 × 10 ³ ▲	0	0	
140 × 10 ³	180 × 10 ³	180 × 10 ³	140 × 10 ³ ▲	100 × 10 ³ ▲	0	0*	0	
97 × 10 ³	170 × 10 ³	240 × 10 ³	1 600 × 10 ³	2 800 × 10 ³	640 × 10 ³	1 0 × 10 ³	40 × 10 ³ ▲	
140 × 10 ³	160 × 10 ³	470 × 10 ³	1 400 × 10 ³	1 600 × 10 ³	800 × 10 ³	400 × 10 ³	87 × 10 ³ ▲	
100 × 10 ³	170 × 10 ³	600 × 10 ³	2 080 × 10 ³	4 000 × 10 ³	5 600 × 10 ³	1 200 × 10 ³	570 × 10 ³	
152 × 10 ³	240 × 10 ³	320 × 10 ³	960 × 10 ³	600 × 10 ³	0	0	0	
116 × 10 ³	140 × 10 ³	570 × 10 ³	3 200 × 10 ³	1 100 × 10 ³	400 × 10 ³	80 × 10 ³ ▲	0	
48 × 10 ³	60 × 10 ³	60 × 10 ³	80 × 10 ³	40 × 10 ³ ▲	40 × 10 ³ ▲	0 × 10 ³ ▲	0	
97 × 10 ³	100 × 10 ³	160 × 10 ³	120 × 10 ³	80 × 10 ³ ▲	20 × 10 ³ ▲	0	0	
4 × 10 ³	54 × 10 ³	97 × 10 ³	240 × 10 ³	2 400 × 10 ³	80 000 × 10 ³	120 000 × 10 ³	64 000 × 10 ³	
132 × 10 ³	180 × 10 ³	400 × 10 ³	800 × 10 ³	3 600 × 10 ³	1 040 × 10 ³	100 × 10 ³ ▲	40 × 10 ³ ▲	
136 × 10 ³	140 × 10 ³	180 × 10 ³	160 × 10 ³	100 × 10 ³ ▲	36 × 10 ³ ▲	0	0	
128 × 10 ³	160 × 10 ³	400 × 10 ³	180 × 10 ³	120 × 10 ³ ▲	0	0*	0*	
117 × 10 ³	157 × 10 ³	840 × 10 ³	4 000 × 10 ³	3 600 × 10 ³	800 × 10 ³	42 × 10 ³ ▲	10 × 10 ³ ▲	
140 × 10 ³	197 × 10 ³	640 × 10 ³	2 000 × 10 ³	1 100 × 10 ³	600 × 10 ³	100 × 10 ³ ▲	0	
97 × 10 ³	170 × 10 ³	120 × 10 ³	80 × 10 ³ ▲	10 × 10 ³	0	0	0	

those in Trypticase soy broth. Contrary to the findings Galask & Snyder (4) showed inhibition of *E. coli* growth by amniotic fluid but the rate of growth was measured by recording turbidometric (spectrophotometrically) of the inoculated samples. Furthermore Bergman et al (1) present an inhibitory activity of the amniotic fluid of *E. coli* in 66.6% of the samples tested. Latzmeier et al (7) using direct viable counts showed a lack of *E. coli* growth in clear amniotic fluid while the addition of 0.5 ml of trypticase soy broth supported a normal rate of growth with a growth curve nearly identical to that in the trypticase soy broth. In the present investigation direct viable counts were performed. Clear amniotic fluid BHI solution and amniotic fluid plus BHI used as media for the growth of *E. coli* revealed results obtained a much lower growth curve of *E. coli* in clear amniotic fluid

which became static in 24 hours in all cases while afterwards a permanent inhibition was noticed (bacteriostatic activity) until 48 hours when in 19 samples a bactericidal effect was observed.

In BHI and Ringer solution which were used as controls the mean growth curves of *E. coli* were nearly identical. Besides the addition of a small amount of BHI to the amniotic fluid enhanced the growth of *E. coli* in it but the curve was lower in comparison with the two controls.

The results of this series agree partly with those of Latzmeier (7) who suggested that growth inhibition of *E. coli* by amniotic fluid is due to the absence of nutrients. The main difference between the results of the present study and those of other authors is that the inhibitory effect of the amniotic fluid started in a few cases after six hours of inoculation and continued in such a rate that after 48 hours 26 of the 29 samples presented a high degree of inhibition with predominance of a bactericidal effect (19 samples).

The presented data support the view that there is a permanent antibacterial activity in amniotic fluid upon *E. coli* growth in vitro which perhaps exists in a similar way in vivo thus protecting both mother and fetus from infection especially after bacterial invasion into the amniotic cavity during pregnancy and labor

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EFFECT OF A COPPER AND A PLASTIC IUD ON THE HISTOCHEMISTRY OF ENDOMETRIAL ENZYMES IN THE RABBIT DURING EARLY PSEUDOPREGNANCY

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The influence of a copper and a plastic IUD on alkalization and intensity of alkaline and acid phosphatase and beta-glucuronidase activity in the rabbit endometrium during early pseudopregnancy was investigated. The activities of the two lysosomal enzymes acid phosphatase and beta-glucuronidase were increased in the epithelial cells in the presence of both types of IUD. The alkaline phosphatase activity was almost entirely abolished in surface and glandular epithelial uterine horns.

Two types of inert IUDs have been found to interfere with the activity of endometrial enzymes. In the presence of inert IUDs alkaline phosphatase activity in human endometria was found to be normal (14, 27) or increased (16, 18). Increased activity of alkaline phosphatase was found in wearers of the Cu T (13). In a study of homogenates of the rat uterus the activity of alkaline phosphatase was increased in the presence of surgical silk or a copper IUD (5, 6). In the non pregnant rabbit it was unaltered in the presence of "inert" IUDs (19, 21). In the rat acid phosphatase activity was found to be increased in the presence of "inert" and copper IUDs in the human (13, 18, 20). Hester et al (16) did not find the normal increase in this enzyme activity at the time of ovulation in the presence of inert IUD. Increased activity of acid phosphatase was found in the rat in the presence of inert and plastic IUDs (5, 6, 17, 20) and in the rabbit in the presence of a copper IUD (7) but not of an inert

IUD. In wearers of the Cu T the activity of beta-glucuronidase was somewhat depressed (13) whereas it was reported to be increased in the rat in the presence of both surgical silk and copper (5, 6). Six of the above cited studies are histochemical (14, 16, 19, 21 and 27).

In our previous studies the DNA synthesis and the morphological development of the endometrium (9, 10, 11) were markedly inhibited in the presence of a copper IUD during early pseudopregnancy and early pregnancy in the rabbit. As it has been suggested that alkaline phosphatase, acid phosphatase and beta-glucuronidase are of importance for the implantation process in this animal (1, 8, 12, 24) the effect of a plastic and a copper IUD on the activity of these enzymes was studied in the same experimental system.

MATERIAL AND METHODS

Virgin rabbits of mixed breed weighing 2-3 kg were used. Two types of IUDs were studied: 1) Copper IUD—consisting of a 50 mm long polyethylene catheter 0.5 mm in outer diameter (Clay Adams Parsippany N.Y. USA) around which a copper wire 0.23 mm in diameter was coiled giving a surface area of 200 mm² (Cu IUD). 2) Plastic IUD—consisting of a polyethylene catheter 1.0 mm in outer diameter and 50 mm long (PI IUD).

The IUDs were sterilized by soaking in ethanol and rinsed in sterile physiological saline before use. The animals were anaesthetized with Nembutal and the abdominal cavity was exposed under sterile conditions via a lower abdominal midline incision. No corpora lutea were present at the time of operation. The IUDs were introduced into the uterine horns via a small puncture in the antimesometric part of the uterine wall and fixed by a

Table I Presentation of various experimental groups with number of animals and main effects of types of IUDs

Group	Combinations of IUDs	Subgroups (hours)	No of animals	Main effects of PI IUD _{1,2} and Cu IUD on the activity of alkaline phosphatase, acid phosphatase and glucuronidase
I	PI IUD _{1,2} -SH	0 120	6 6	<p>Alkaline phosphatase In PI IUD_{1,2} exposed horns at 0 hours essentially the same appearance as for sham-operated horns (see text)</p> <p>Acid phosphatase In PI IUD_{1,2} exposed horns at 0 hours increase in the activity of the enzyme in the apical surface epithelium. At 120 hours no difference from sham-operated horns</p> <p>Beta glucuronidase In PI IUD_{1,2} exposed horns at 0 hours more intense staining in the surface epithelial cells. At 120 hours intensively stained inflammatory cells. At 120 hours no difference from sham-operated horns</p>
II	PI IUD _{1,2} -Cu IUD	0 120	6 6	<p>Alkaline phosphatase In PI IUD_{1,2} exposed horns at 0 hours see above for Group I. In Cu IUD-exposed horns at 0 and 120 hours an almost completely absent activity in surface and glandular epithelial cells. As a number of inflammatory cells with an intense enzyme activity.</p> <p>Acid phosphatase In PI IUD_{1,2} exposed horns at 0 and 120 hours see above for Group I. In Cu IUD-exposed horns at 0 and 120 hours no difference from PI IUD_{1,2} horns. At 120 hours as in PI IUD_{1,2} horns</p> <p>Beta glucuronidase In PI IUD_{1,2} exposed horns at 0 hours see above for Group I. In Cu IUD-exposed horns at 0 and 120 hours no difference from PI IUD_{1,2} horns. At 120 hours increased activity in surface epithelial cells especially at the minor folds. Strong enzyme activity in inflammatory</p>

4-0 silk suture at the site of the puncture. Uterine horns designated as sham-operated (SH) were exposed to introduction and withdrawal of the PI IUD_{1,2} 4-0 silk suture being placed at the site of the puncture. The effect of the Cu IUD was compared with that of the PI IUD_{1,2} in the same animal. One horn was fitted with the PI IUD_{1,2} and the other with the Cu IUD except in the group where one of the horns was sham-operated. The IUDs were always randomized between the horns.

On the fifth day after operation most of the animals were injected with HCG—75 IU (HCG, human chorionic gonadotropin, Gonadex, Leo AB, Helsingborg, Sweden) intravenously to induce pseudopregnancy. Some animals killed on the fifth day after operation were not injected with HCG (0 hours). Table I lists the experimental groups. The animals were killed by cervical dislocation at 0 and 120 hours after injection. The internal genitalia were taken out and the uterine horns were trimmed. The presence of corpora lutea in each ovary was registered. The middle part of the length of the uterine horn exposed to the IUDs was used for enzyme studies.

The tissue was immediately frozen in propane cooled by liquid nitrogen and preserved at -70°C until further processed. Cryostat sections were fixed either in 10% formalin (containing 0.07 M phosphate buffer, pH 7.4) for 10 min or in absolute acetone at -20°C for 15 min and for

another 15 min at room temperature. The formalin sections were rinsed for 5 min in 0.1 M phosphate buffer (pH 7.4).

The acetone fixed sections were stained for alkaline phosphatase and beta glucuronidase. The reaction was carried out according to azo-dye methods with AS-TR phosphoric acid (sodium salt) as substrate for the two phosphatases (3, 4) and naphthol AS-BI-gluc for the beta glucuronidase (15). The tissue sections were incubated for 30 min for the alkaline phosphatase (pH 9.1) and for 60 min for the acid phosphatase and beta glucuronidase reaction (pH 5.2). The procedures were checked with simultaneous sections of rat liver sections which showed a normal pattern.

RESULTS

Alkaline phosphatase activity

The normal development of the endometrium after HCG injection was essentially the same as described previously (11) to judge from sections of sham-operated horns stained for alkaline phosphatase and studied at 0 and 120 hours.



The alkaline phosphatase activity in the apical part surface epithelial cells of a PI IUD₁₀-containing horn (a) is almost completely abolished in the Cu IUD-

containing horn (b). The comparison is made between horns within the same animal.

The alkaline phosphatase activity in the endometrium of unstimulated (0 hours) sham-operated horns (in animals where the other horn was fitted with the PI IUD₁₀) was localized as a granular line in the apical part of most of the surface and glandular epithelial cells. There was an intense activity just beneath the epithelium in the endothelial lining of the stromal vessels.

120 hours after HCG stimulation the alkaline phosphatase activity was markedly increased and localized in the apical part of the surface epithelial cells. In addition, an intense activity in the stromal septa of the minor folds was observed (Fig. 1A). The activity of the glandular epithelial cells was generally faint. In some areas activity could be found. In the subepithelial part of the glands the activity was generally faint. The activity of the endothelial cells of the vessels had the same appearance as that found in unstimulated rabbits.

The range of the alkaline phosphatase activity in the presence of the PI IUD₁₀ was essen-

tially the same as that described above for sham-operated horns, irrespective of the treatment of the contralateral horn: sham-operation or exposure to the Cu IUD.

In horns fitted with the Cu IUD the alkaline phosphatase activity was almost completely abolished in surface and glandular epithelial cells at 0 and 120 hours (Fig. 1B). Furthermore, there was a markedly increased number of inflammatory cells with an intense alkaline phosphatase activity. These cells were found subepithelially and in the uterine lumen.

Effect of cupric ions on the alkaline phosphatase reaction

As the alkaline phosphatase activity was almost completely abolished in endometrial cells exposed to the Cu IUD, sections from sham-operated horns (120 hours after HCG injection) were stained for alkaline phosphatase in the presence of cupric ions (cupric acetate-*pro analysi*) added to give final cupric ion concentrations from 3×10^{-4} to

$3 \times 10^{-2} M$) Cupric ions at these concentrations were found to reduce the alkaline phosphatase staining during the first 15 min of incubation. However the intensity of staining after 30 min of incubation was the same as in control sections where no cupric ions were added to the reaction mixture.

Acid phosphatase activity

A faint and mainly granular acid phosphatase activity was seen in the apical part of the surface and glandular epithelial cells in sham operated horns at 0 hours. Faint acid phosphatase activity was also found in the endothelium of stromal vessels and in scattered stromal cells.

At 120 hours no activity could be found in most surface epithelial cells. The glands showed essentially the same activity as described above at 0 hours. The endothelial cells in the stromal vessels and scattered stromal cells showed a faint enzyme activity. There was a tendency to an accumulation of the stromal staining beneath the surface epithelium.

The endometrium of horns fitted with the PI IUD₁₀ showed the same localization and intensity of acid phosphatase activity as sham-operated horns except at 0 hours when the activity of the apical part of the surface epithelium was increased. The PI IUD₁₀ had no obvious effect on the staining of the glandular epithelial cells. The same picture was obtained in the presence of the PI IUD₁₀ irrespective of the type of treatment of the contralateral horn—sham-operation or exposure to the Cu IUD.

No difference in the acid phosphatase activity could be found in the endometrium of horns fitted with the Cu IUD or the PI IUD₁₀ in unstimulated animals. At 120 hours areas of more intense enzyme activity mainly localized in the surface epithelium were found in horns exposed to the Cu IUD.

Beta glucuronidase activity

In sham-operated horns at 0 hours enzyme activity was seen in the surface and glandular epithelial cells with a more pronounced activity in the apical part of the cells. Activity was also found in inflammatory cells lying in the stroma.

At 120 hours the activity of this enzyme was generally weaker being mainly localized in the

basal part of the surface epithelial cells and in the apical part of the glandular epithelial cells.

At 0 hours horns fitted with the PI IUD₁₀ showed a more intense staining in the apical part of the surface epithelial cells. Furthermore an increased number of stained (probably inflammatory) cells lay in the stroma. At 120 hours there was a definite difference between PI IUD₁₀-containing horns and sham-operated horns.

The appearance of the beta glucuronidase activity in horns fitted with the Cu IUD studied at 0 hours was the same as described above for horns containing the PI IUD₁₀. At 120 hours the surface epithelial cells of the Cu IUD-containing horns showed an increased enzyme activity especially in areas at the top of the minor folds. There were also stained inflammatory cells in the stroma, which accumulated beneath the surface epithelium.

DISCUSSION

The appearance of the enzyme activity (alkaline phosphatase and acid phosphatase and beta glucuronidase) in sham operated horns of pseudopregnant animals studied in the present investigation agrees well with that described by Petry et al (24) for normal horns.

Different kinds of IUDs have been found to interfere with the activities of several endometrial enzymes among these the most frequently studied are alkaline phosphatase, acid phosphatase and beta glucuronidase. These enzymes are of special interest; they are supposed to participate in the implantation process (1, 8, 12, 24). Furthermore acid phosphatase and beta glucuronidase are lysosomal enzymes and copper is known to accumulate in the lysosomes (2); therefore the activity of these enzymes might be altered in the presence of a copper IUD and possibly also in the presence of inert IUDs as these have been found to cause an increase in the copper concentration in the cervical fluid of women (26).

Alkaline phosphatase activity in human endometrial surface epithelial cells increases as the endometrium grows and reaches a peak during the late proliferative stage (22). This enzyme needs the presence of zinc for optimum activity (23) as discussed by Zipper (29) might therefore be affected by the presence of other cations, e.g. cupric ions.

ter" IUDs have generally been found to in-
crease the acid phosphatase activity both in rats (5
'70) and in women (18). In rabbits the activity
of this enzyme was reported to be unaltered (21).
slightly increased epithelial activity in the
presence of the PI IUD₁₀ described in the present
study is at variance with the latter finding.

Copper IUDs were reported to cause an in-
creased activity of acid phosphatase in various
species (2, 6, 13). Similar results were obtained in
the present study.

In the present investigation the epithelial activity
of β -glucuronidase was increased and a greater
number of positive inflammatory cells appeared in
the presence of both types of IUDs—especially in
the case of the Cu IUD. In homogenates a greater
activity was reported from studies in the rat (5, 6).
However, in women wearing the Cu T the activity
of this enzyme was found to be slightly decreased.

As polymorphonuclear leucocytes are rich in
 β -glucuronidase the different results might be
due to differences in the number of inflammatory
cells (cf. 7, 13).

In the rat both a copper and an inert IUD
resulted in a higher uterine alkaline phosphatase
activity (5, 6). This activity was found to be
decreased (14, 19, 21, 27) or increased (16, 18) in the
presence of inert IUDs in different species. The
same in women caused a decreased activity (13).
The results agree well with the latter finding, as the
alkaline phosphatase activity of epithelial cells was
not completely abolished in the presence of a
plastic IUD. The present study found that a plastic
IUD had no influence on the activity of alkaline
phosphatase. Again, some of the differences in the
results of various reports might be explained by the
use of leucocyte alkaline phosphatase activity
histochemical determinations.

Wilson (28) has demonstrated a reduced activity
of human endometrial alkaline phosphatase in
homogenates both in the presence of a copper foil
and cupric ions. This agrees with our finding that
alkaline phosphatase staining during the first 15
min of incubation was reduced in the presence of
cupric ions. However, after 30 min of incubation
alkaline phosphatase staining in the presence of
cupric ions was identical with that in control
homogenates. The alkaline phosphatase staining is thus
inhibited by cupric ions, but probably not enough to
cause the complete loss of activity in the surface
epithelial cells of horns exposed to the copper IUD.

in vivo. Thus there might be a lack of this enzyme in
the epithelium which can be due to a differentiation
defect.

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PROGNOSIS RECURRENCES AND METASTASES CORRELATED TO HISTOLOGIC CELL TYPE IN CARCINOMA OF THE UTERINE CERVIX

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Abstract An analysis of 230 patients treated with radiotherapy for cervical cancer stages I and II is presented. No correlation to cell type was found as regards the number of failures recurrence rate or the frequency of local or distant metastases. Although different biological behavior might be shown by cancers of varying cell type adequate planning and staging before treatment seems to be of better prognostic value.

The present investigation correlates death from cervical cancer and number of recurrences and metastases to regional lymph nodes and distant organs with the three groups of histopathologic cell differentiation as suggested by Reagan et al 1957 (18).

MATERIAL AND METHODS

Case material is comprised of 230 women with stages I and II carcinoma of the uterine cervix according to FIGO 1970 (24) that were treated during 1969 and 1970 in the Gynecologic Section Department of Radiation Therapy University Hospital Lund Sweden.

Patients were treated according to the following principles. External high voltage irradiation later supplemented by intracavitary treatments was given to most of the patients. Exceptions were made in patients with small and early tumors they being given only intracavitary treatment in two sessions 3 weeks apart. When combined external and intracavitary treatment was chosen only one application was given. The interval between external and intracavitary treatment was three weeks.

External irradiation was given with ^{60}Co and 33 MV photons dependent upon the size and configuration of the patient. The pelvis was given 850-950 rad each week in five fractions using two opposing fields. The field size was determined from the size of the pelvis. Both fields were treated daily.

Point A (7 cm lateral and 2 cm cranial from the external os of the uterine cervix) is the reference point for given absorbed dose. The external treatment was supposed to deliver a dose between 3 000-4 500 rad at point A.

Each intracavitary treatment lasted 12-20 hours. At point A the dose rate was 150-200 rad/h according to type of applicators used. These have been described previously (10). They can be locked in a definite position which implies that the dose measured in point A is representative for the whole treatment.

car of uterine cervix is in its early stages successfully treated both by surgery and/or radiotherapy. Several attempts have been made to select treatment according to the cytologic and histologic classification made upon smears or biopsies from the tumour (2-6). Varying results have been obtained but so far no consistent guidelines as regards the future treatment of the patient can be based on the morphologic picture. This seems to be relevant in addition to squamous cell cancer of the uterine cervix when subclassified according to criteria presented by Reagan et al 1957 (18). Thus some investigators have found that patients with squamous cell cancer of small cell type as well as patients with keratinizing squamous cell cancer have less chance of survival when compared with a group of patients having squamous cell cancer of non keratinizing type when all were treated by radiotherapy (16-22). However others were not able to demonstrate any difference in survival or the frequency of recurrences among patients treated by radiotherapy stating that surgery for stages I and II squamous cell carcinoma of the cervix will have to be justified on a basis other than tumour grade (7).

Table I Distribution of 230 patients with uterine cervical cancer according to cell type and stage 1969-1970

Cell type	Stage 1	Stage 2	Total no
Non keratinizing ca	107	68	170 (74%)
Keratinizing ca	13	22	35 (15%)
Small cell ca	0	6	6 (3%)
Adeno-squamous ca	1	4	5 (2%)
Adenocarcinoma	1	7	8 (4%)
Total	123	107	230 (100%)

The total dose at point A from external and intracavitary irradiation varied between 5000-7000 rad dependent on the size and extension of the tumor and the dose estimated to be received by the urinary bladder and rectum. When calculating the external and intracavitary dose no correction has been made for the difference in biologic effects due to different fractionation scheme (4). Patients having an adenocarcinoma were given 3000-4500 rad to the pelvis as described above and six weeks after radiotherapy a total hysterectomy including a vaginal cuff was performed (9). According to the findings at operation supplementary radiotherapy was given afterwards.

In a few cases the anatomy of vagina and cervix did not permit the use of the radium applicators as described above. Then supplementary external treatment up to 6500 rad was given to the primary tumor about 3 weeks after the initial series of treatment (21 patients). Occasionally intracavitary applicators were used which do not permit adequate dose estimation (5 patients).

The histopathologic classification relies on biopsy specimens, small for diagnostic purposes and cone biopsies made in an attempt to excise the tumor. In patients with an adenocarcinoma there is a post therapeutic hysterectomy specimen which also is available in many patients with recurrences of the squamous cell variety.

Five different groups were used: squamous cell cancer of small cell type, of keratinizing large cell type, of non keratinizing large cell type, pure adenocarcinoma and adeno-squamous cancer. In specimens with transitional forms or of mixed types the dominant pattern has decided

Table II Death from cancer of 230 patients with uterine cervical cancer according to cell type and stage during an observation period of 3.5-5.5 years

Cell type	Stage 1	Stage 2	Total no
Non keratinizing ca	4/107	16/68	20/170 (12%)
Keratinizing ca	3/13	5/22	8/35 (~23%)
Small cell ca	0	3/6	3/6
Adeno-squamous ca	0/1	2/4	2/5
Adenocarcinoma	0/7	4/7	4/14
Total	7/123	30/107	37/230
	(6%)	(28%)	(16%)

Table III Frequency of recurrences in 219 patients with uterine cervical cancer in relation to cell type and stage during an observation period of 3.5-5.5 years

Cell type	Stage 1	Stage 2	Total no
Non keratinizing ca	3/102	9/68	12/170
Keratinizing ca	7/13	4/22	11/35
Small cell ca	0	2/6	2/6
Adeno-squamous ca	0/1	3/4	3/5
Adenocarcinoma	0/7	7/7	7/14
Total	5/123	23/107	28/230
	(4%)	(19%)	(11%)

the classification but a clarifying notation is added when the statistical results are evaluated.

All patients have been observed between 2.5-5.5 years. The diagnosis of recurrences and/or metastases was made by physical and radiologic examination, curettage and biopsies. The patients have not been observed for progression of residual tumor or recurrences (4). When recurrent tumor growth or metastases were detected after therapy was finished only the first site has been recorded.

RESULTS

In Table I the histopathological diagnoses are grouped with all patients grouped in stage I or stage II. A significantly lower number of large cell non keratinizing cancers was found in stage II in comparison with stage I ($p < 0.05$).

The proportion of patients who died from disease is somewhat higher among women with large cell keratinizing cancer as compared to those with large cell non keratinizing squamous cell cancer. The difference however is not statistically significant and is only observed in stage I patients.

Table IV Frequency of lymph node metastases in 230 patients with uterine cervical cancer in relation to cell type and stage during an observation period of 3.5-5.5 years

Cell type	Stage 1	Stage 2	Total no
Non keratinizing ca	7/107	7/68	14/170
Keratinizing ca	0/13	0/22	0/35
Small cell ca	0/0	0/6	0/6
Adeno-squamous ca	0/1	0/4	0/5
Adenocarcinoma	0/7	1/7	1/14
Total	2/123	4/107	6/230
	(1%)	(4%)	(3%)

e V Frequency of distant metastases in 230 patients with uterine cervical cancer in relation to type and stage during an observation period of 5.5 years

type	Stage I	Stage 2	Total no
keratinizing ca	0/102	6/68	6/170 (4%)
nonkeratinizing ca	1/13	0/22	1/35 (~3%)
large cell ca	0/0	2/6	2/6
non-squamous ca	0/1	0/4	0/5
squamous carcinoma	0/7	1/7	1/14
I	1/123 (1%)	9/107 (8%)	10/230 (4%)

number of patients in these particular groups is too low to permit a conclusive statistical evaluation (Table II). Among stage I patients with large cell nonkeratinizing cancer one did not receive adequate curative treatment which resulted in a local recurrence that was not possible to cure. One patient with fatal large cell non keratinizing cancer given external radiotherapy only. In Tables III and V are given the frequency of recurrences. The number of patients with metastases to regional lymph nodes and to distant organs are tabulated. Significant differences can be found statistically. The number of positive observations in each group is too low to permit statistical evaluation. There is a tendency to a lower frequency of recurrence and a higher percentage number of metastases to regional lymph nodes among women with large cell nonkeratinizing squamous cell carcinoma. Among the 25 patients with recurrences 12 were seen in the group of non keratinizing large cell carcinoma. Seven out of these twelve patients were found to have a poorly differentiated squamous cell cancer with large occasionally bizarre tumor cells demonstrating a total absence of keratinization. These cancers do not fulfil the criteria of a small cell carcinoma but using common histologic criteria must be regarded as poorly differentiated carcinomas (1).

DISCUSSION

For a long time treatment of early stages of invasive carcinoma of the uterine cervix has been a controversial matter. Some advocate a surgical procedure as the most effective (17) while others favor radiotherapy as the initial choice. The results in these series seem to be equally good and comparable

both in regards to cure rate and complication rate (3 12 14 15 19 21).

Several attempts have been made to correlate histologic cell type with the prognosis of the patient with a uterine cervical carcinoma. Some authors were not able to find such a correlation (13) while other results (23) indicate a favorable prognosis in patients with non keratinizing squamous cell cancer in comparison with cancer of other cell types. However it is not satisfactory to rely on the cure rate only when the efficiency of a particular treatment is to be evaluated. All separate factors influencing the prognosis have to be considered. Among these the initial treatment of the primary tumor has to be studied in particular but in addition local spread of the tumor as well as metastases to regional lymph nodes are of interest as these areas can be effectively treated with various techniques. For distant metastases no curative treatment can be offered at present.

Most series are presented in an attempt to correlate the histopathology to the survival rate. The results so far have been controversial (7 16 22 23).

Attempts have been made to correlate the histopathologic picture to both local failures and all other treatment failures (7). No statistically conclusive evidence has been obtained mainly due to the low number of such failures.

An attempt to evaluate the prognostic value of the histopathological classification is not possible unless the patients have well-defined tumors which display a limited spread i.e. stage I or II. In addition a biopsy from more advanced tumors is less likely to be representative. The more advanced tumor in addition generally offers greater therapeutic problems and an estimation of the delivered dose might be more difficult to calculate due to patient and tumor-dependent variables in each case.

The technique applied is of decisive importance in order to obtain favorable results in a given patient. Some previously reported failures might be explained by inadequate radiotherapy (7). However the use of modern radiotherapy with intracavitary applicators does not imply that the estimated dose can be used as an indicator of radiosensitivity or even radio-curability. Several difficulties are apparent when the dose given to the tumor area is calculated even if locked or combined applicators are used (10 20). When separate intra uterine and intravaginal applicators are preferred

this estimation is practically impossible. The use of point A as representative for absorbed dose is possible only if the distribution of radiation around each applicator combination is known in detail. In the present series of patients the dose at point A varied between 5000–7000 rad while the absorbed dose in the central part of the uterine cervix was estimated to vary between 9000–16000 rad. When external radiotherapy is given alone, however, a well defined and even dose is applied to the tumor area.

In 21 patients in this series receiving external therapy solely, the absorbed dose is considered to lie within $\pm 10\%$ of the calculated dose. This has been confirmed by dose measurements *in vivo* that are routinely made in all patients (11). Among these 21 patients 17 had squamous cell cancer. Of these 12 were of large cell non keratinizing type and in this group 3 recurrences were observed. The remaining five patients were of large cell keratinizing type and here no recurrences were observed.

The statistical analysis could not reveal a positive correlation between histological cell type and recurrence rate, number of failures of radiotherapy and frequency of metastases to regional lymph nodes or distant organs. Although the number of patients in each group is small, which makes a statistical analysis less reliable or even impossible, the results obtained may be explained in at least two different ways.

1. There is no apparent difference in biological behavior between cancer of different cell types. This explanation seems less plausible as a number of observations indicate several points of difference in behavior (1, 16, 18).

2. The radiotherapy administered as described above is equally effective for all types of cancers. A failure is not dependent on cell type and biological behavior but on inadequate staging before treatment or factors persistent in a given patient such as abnormal anatomical conditions and/or inadequate positioning of intracavitary applicators—factors obviating an optimal treatment.

CONCLUSION

This investigation has not been able to demonstrate significant prognostic differences allowing statistical analysis between the two histopathological types of squamous cell cancers (large cell keratinizing and large cell non keratinizing squamous

cell cancer). Thus a pretreatment classification of histologic subgroups has been of limited value regarding planning the treatment of the patient. In order to improve the results an analysis is in progress of all technical aspects of the treatment as well as accurate preliminary staging seems to be more beneficial (8).

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VALUE OF ULTRASONIC DIAGNOSIS OF GYNECOLOGICAL TUMORS IN 370 SURGICAL CASES

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Abstract Ultrasonic exploration of the female pelvis to confirm or supplement the usual clinical examination enabled a correct diagnosis to be made in 80% of the 370 cases examined and subsequently operated on. The echographic description always included the size of the tumor, its cystic or solid nature and its site of origin. Out of 3 uterine and ovarian tumors measuring less than 3 cm, 15 were discovered.

Uterine tumors were revealed by means of ultrasound as early as 1958 by Donald (2) since then the two-dimensional echographic aspects of gynecological tumors have been described frequently (3-14). The value of echographic diagnosis of pelvic masses in gynecology was assessed from a series of 69 patients by Levi in 1971 (9). That study has been pursued further and the cases of 370 women operated on after ultrasonic examination are reported in this paper. The ultrasonic technique and the method used to interpret the echograms were published earlier (9-10).

MATERIAL AND METHOD

From 1968 to March 1974, 8112 patients were examined at the Ultrasonic Diagnosis Department of the Clinic for Obstetrics and Gynecology, Hôpital Universitaire Brugmann, Brussels. 519 examinations had been requested to confirm or complement the clinical diagnosis of these 519 patients. 370 later underwent surgery. The preoperative macroscopic and microscopic description of the genital organs was then compared with the preoperative echographic description in order to check the ultrasonic diagnosis.

The report on the echogram contains details which permit: (1) detecting the presence or absence of a pelvic mass; (2) defining its nature (solid, cystic or complex tumor with solid tissues and liquid areas); (3) measuring the tumors; and (4) pinpointing their origin as closely as possible (uterus, ovaries, Fallopian tubes, etc.).

The macroscopic and microscopic diagnoses served as a reference or final diagnosis. The ultrasonic diagnosis (USD) was classified as:

(a) *correct* if it corresponded to the reference diagnosis in every respect;

(b) *doubtful* if it had been impossible to choose between several diagnoses provided that the various possibilities put forward included the true diagnosis;

(c) *false positive* if a non-existent tumor was described on the echogram;

(d) *false negative* if an existing tumor was not observed on the echogram; and lastly

(e) *mistaken* if one tumor was mistaken for another (uterine leiomyoma instead of a solid tumor of the ovary, for instance).

The same method was used to analyze the value of the clinical diagnosis (CD). It should be noted that the clinical diagnoses mentioned were generally made after the usual clinical examination and in most cases before any further exploration.

RESULTS

The breakdown of the lesions defined according to the operative and microscopic descriptions, such as leiomyoma, cyst, polyp, is shown in detail in Table I, together with the number of correct, mistaken, doubtful, false positive and negative diagnoses from both clinical and ultrasonic examinations.

In all, the ultrasonic diagnosis (USD) is fully correct in 80% of the cases, doubtful in 5%, mistaken in 6%, false positive in only 2% and false negative in 7%.

Regarding the size of the tumors found during laparotomy (Table II), it will be remarked that only tumors of over 2 cm were successfully interpreted by ultrasound. Apparently, therefore, masses of less than 3 cm cannot be distinguished by ultrasonic exploration. In view of the high frequencies applied (2-10⁶ Hz), it is not a question of resolution power.



Fig. 1 Uterine leiomyoma (F) (12×11×11 cm) at least one subserous fibroid (f) is visualized to the right on transverse scan. High signals amplification but weak echos from tumor distal boundary (arrow)



Fig. 4 Polycystic ovary (9×7.5×6 cm)



Fig. 2 Uterine leiomyoma (12×6×9 cm) with edematous degeneration: echos inside and cystic areas (arrow)



Fig. 5 Dermoid cyst in the Douglas pouch (typical lar pattern left; cystic pattern right)

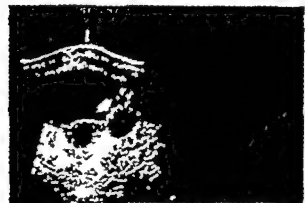


Fig. 3 Ovarian cyst (3×3×3 cm) (arrow)



Fig. 6 Cyst adenocarcinoma (17×9×7 cm) bipolar and polypoid patterns



Fig 7 Ovarian carcinoma (9x7x7 cm) ascites (A) and boneal metastasis (M)



Fig 9 Bilateral pyosalpinx (3x2x2 cm) (arrows)

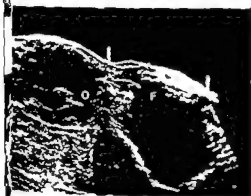


Fig 8 Uterine leiomyoma (F) and polycystic ovary (O) show differences in sound attenuation by the amount of blood behind the two different kinds of tumors



Fig 10 Tubal pregnancy (arrow) and blood collection in Douglas pouch (D)

On the other hand the tissues surrounding the uterus and Fallopian tubes (parametrium, broad ligament etc.) are liable to disguise small swellings which are sometimes visible on the echogram but which had to be interpreted. As regards the small leiomyomata many of them were in intra-uterine positions and others were subserous. Possibly they escaped the examiner because the tumors were too far apart. Thus if one disregarded tumors measuring less than 3 cm the rate would be an excellent level of 98% correct ultrasound diagnoses in the case of leiomyomata and ovarian cysts. These observations likewise relate to tubal pregnancies. It would be interesting to detail the clinical circumstances for the 70% of mistaken doubtful or false positive or negative diagnoses. We shall confine ourselves to a brief comment on Tables III

IV and V which tabulates the clinical and ultrasonic diagnoses in a double entry form

(a) the two mistaken clinical diagnoses (CD) were pregnancy of about three months

(b) the 8 leiomyomata were clinically diagnosed five times as an ovarian cyst and three times as a pregnancy; ultrasound read them eight times as a normal uterus

(c) in these two cases the leiomyomata measured less than 3 cm and the pelvic organs were judged to be normal by ultrasound

(d) the CD was leiomyoma

(e) the ultrasonic diagnosis (USD) was ectopic pregnancy six times and fluid filled tumor twice; the CD was leiomyoma (7 cases) and ectopic pregnancy (1 case)

Table I *Ultrasonic and clinical versus pathology findings*

Organs	Pathology findings	No of cases	Ultrasonic findings					Clinical findings			
			Cor rect	Mis taken	False +	False -	Doubt ful	Cor rect	Mis taken	False +	False -
Uterus	Leiomyoma	152	142	0	0	10	0	76	10	0	0
	Polyps	4	2	0	0	1	1	0	2	0	0
	Adenocarcinoma	6	0	2	0	0	4	0	5	0	0
	Pregnancy	1	1	0	0	0	0	0	1	0	0
	Total	163	145	2	0	11	5	76	8	0	0
	%	100	88.96	1.23	0	6.75	3.07	46.63	4.91	0	0
Ovaries	Cyst	114	96	12	0	5	1	35	12	0	0
	Solid tumor	22	14	3	0	3	2	0	6	0	3
	Cystadenocarcinoma	12	12	0	0	0	0	0	0	0	0
	Total	148	122	15	0	8	3	35	18	0	3
	%	100	82.43	10.14	0	5.41	2.03	23.65	12.16	0	2.03
Tubes	Ectopic pregnancy	27	10	6	0	2	9	10	5	0	2
	Pyohydrosalpinx	11	7	0	0	3	1	3	7	0	0
	Total	38	17	6	0	5	10	13	12	0	2
	%	100	44.74	15.79	0	13.16	26.32	34.21	31.58	0	5.26
Miscellaneous	Ascites	6	5	0	0	1	0	1	2	0	1
	Intestinal obstruction	1	0	0	0	1	0	0	1	0	0
	Normal pelvis	14	7	0	7	0	0	0	0	14	0
	Total	21	12	0	7	2	0	1	3	14	1
	%	100	57.14	0	33.33	9.52	0	4.76	14.28	66.67	4.76
Total		370	296	23	7	26	18	125	41	14	16
	%	100	80.0	6.2	1.9	7.0	4.9	33.8	11.1	3.8	4.3

(f) the USD was ectopic pregnancy (3) and hydrosalpinx (1) while the CD hesitated between tumor and ectopic pregnancy (3) and between leiomyoma and cyst (1)

(g) normal pelvis according to ultrasound

(h) in all three cases ultrasound interpreted the pelvis as normal while the clinical diagnosis was leiomyoma (2) and an ectopic pregnancy (1)

(i) the pelvis was normal on ultrasound whereas

the clinical interpretation was cyst ectopic pregnancy mass

(j) neither ultrasound nor the clinical distinguished between a subserous leiomyoma and an ovarian cyst

(k) in every case there was excellent agreement between the CD and USD ectopic pregnancy ascites (1) pyosalpinx (?)

(l) in all 9 cases including solid ovarian tumor

Table II *Tumor size*

Ultrasonic diagnosis	Less than 1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm	More than 5 cm	Total
<i>Ovarian cysts</i>							
Correct	0	0	7	6	9	74	96
Error	8	9	1	0	0	0	18
Total	8	9	8	6	9	74	114
<i>Uterine leiomyomata</i>							
Correct	0	0	8	15	13	106	142
Error	4	4	2	0	0	0	10
Total	4	4	10	15	13	106	152

e III Uterine leiomyomata

Ultrasonic diagnosis	Clinical diagnosis					Total
	Cor rect	Mis taken	False +	False -	Doubt ful	
Correct	76	0	0	0	64	140
Mistaken	0	0	0	0	0	0
False +	0	0	0	0	0	0
False -	0	0	0	0	0	0
Doubtful	0	0	0	0	0	0
Total	6	10	0	0	66	15

Text

e IV Ovarian cysts

Ultrasonic diagnosis	Clinical diagnosis					Total
	Cor rect	Mis taken	False +	False -	Doubt ful	
Correct	34	1	0	0	61	96
Mistaken	0	0	0	0	0	0
False +	0	0	0	0	0	0
False -	1	3	0	0	1	5
Doubtful	0	0	0	0	1	1
Total	35	1	0	0	67	114

Text

pyosalpinx (4) ascites (1) and pregnancy (1) were correct and CD mistaken (2 ectopic pregnancies 3 ovarian cysts 4 uterine fibroids)

1) CD was pelvic tumor in 7 cases whereas it was described as normal by ultrasound surgical procedures (laparotomy or laparoscopy)

2) 1 case of moderate ascites was diagnosed by ultrasound and confirmed by laparotomy whereas it was only abnormal pelvis

3) doubtful CD in 28 cases in which USD was correct 12 ovarian cystadenocarcinomas 11 ovarian solid tumors 2 endometrial polyps 2 cases of ascites and 1 pyosalpinx

4) these 11 cases were mistaken as well after ultrasound as by clinical examinations respectively follows 3 ovarian solid tumors 2 cysts and 1 adenocarcinoma (USD) and 3 ovarian cysts 4 ectopic pregnancies 3 ovarian cysts 1 ovarian solid mass (USD) and 3 leiomyomata 1 ovarian cyst (CD) 2 pyosalpinx and 2 endometrial cancers mistaken and described after ultrasound clinical examinations such as 2 ectopic pregnancies and 2 uterine leiomyomata

(r) ectopic pregnancy (2 cases) considered as tubal masses (CD) and as hydro pyosalpinx (USD)

(s) 7 normal cases (laparoscopy) whereas USD was possibility of small cysts (3)—ectopic pregnancy (2) solid ovarian cyst (2) pelvic mass (7) for CD

(t) in 1 case of tubal abscess USD was normal pelvic status CD as correct

(u) USD was no pelvic tumor seen CD was ovarian cyst (1) leiomyoma (1) and pelvic mass (1) when laparotomy and microscopic findings were ascites (1) intestine obstruction (1) and uterine polyp (1)

(v) 2 cases of ectopic pregnancy and 3 cases of solid ovarian tumors were erroneously interpreted as normal pelvis after 1st clinical and ultrasonic examinations

(w) these 6 cases were mistaken (CD) and doubtful (USD) 1 uterine polyp polyp or very early pregnancy (USD) and uterine leiomyoma (CD) 1 ectopic pregnancy and 1 pyosalpinx 2 hydro-pyosalpinx or ectopic pregnancies (USD) and ovarian cysts (CD) 3 adenocarcinomas uterine leiomyomata or cancers (USD) and uterine leiomyomata (CD)

(x) neither USD and CD were able to state precisely the correct diagnosis in these 11 cases 8 ectopic pregnancies 2 solid ovarian tumors and 1 ovarian cancer

The sensitivity of ultrasonic diagnosis or the ability of the examination to produce a positive finding in a genuinely positive situation was calculated

It was also considered useful to test the specificity of ultrasonic diagnosis or its ability to produce a negative finding in a genuinely negative situation In view of the uncertainty caused by the fact that no

Table V Miscellaneous

Ultrasonic diagnosis	Clinical diagnosis					Total
	Cor rect	Mis taken	False +	False -	Doubt ful	
Correct	13 ²	9 ¹	7 ²	1	28	58
Mistaken	0	11	0	0	2	13
False +	0	0	7	0	0	7
False -	1 ¹	3	0	5	0	9
Doubtful	0	6 ²	0	0	11 ²	17
Total	14	29	14	6	41	104

k-x see text

check could be made in 149 cases where surgery was not indicated it is impossible to state that in fact there was no lesion

COMMENTS

It can be seen from analysis of the cases dealt with that exploration by ultrasound is of great value in diagnosing the presence of a tumor in the pelvis particularly where the tumor diameter is greater than 2 cm. In such cases the sensitivity of the echographic description is close to 0.98. It is also very accurate in cases of ascites. The rate of correct ultrasonic diagnosis is lower for ovarian cysts than for uterine leiomyomata (84% against 93%). Furthermore it is also lower for all ovarian tumors than for tumors in uterine origin (82% against 89%). The difference in the results probably stems from the fact that where solid tissue is concerned only two out of three ovarian tumors are correctly diagnosed since this type of tumor is often described as a uterine leiomyoma on the echogram. The high rate of correctly diagnosed cystadenocarcinomas is due to the fact that this type of tumor which usually contains several cystic loculi and numerous vegetations displays a highly characteristic pattern under ultrasound.

As to ectopic pregnancies one can see how essential it is to know the patient's history since in another set of 87 patients (11) examined for specific reasons such as amenorrhea, pain or haemorrhage the ultrasound sensitivity level is 0.82 against 0.37 in the present set.

Thompson et al. have published in 1967 a series of 100 patients with 63 good ultrasonic results (14). Hollander in 1968 has confirmed by ultrasound the presence as the absence of a tumor in 103 of the 114 studied cases (3).

In a very recent study Cochrane & Thomas (1) reported 82% correct diagnoses by ultrasound (80% in our series) but confined themselves to describing the size, nature and uterine or extra uterine location of the lesions observed. It appears however that these authors met with the same difficulties of interpretation more particularly in regard to solid tumors of the ovary. Comparison with the initial conclusions of the clinician shows that ultrasonic examination, comfortable and danger free for the patient, is the ideal complementary exploration for refining a diagnosis.

Ultrasonic exploration misses only 7% of cases where a tumor is present (usually measured by more than 3 cm) and the echogram indicates an existing tumor in less than 2% of the cases. Resorting to other methods of gynecological diagnosis (i.e. X-ray hysterosalpingography, laparoscopy etc.) which still need to be used in difficult cases simply by coupling ultrasonic exploration with the usual clinical diagnosis made in the consulting room, the rate of false results will decrease considerably.

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VIDEO DISPLAY SYSTEM FOR FETAL AND MATERNAL MONITORING

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For Signals from different fetal and maternal
ring equipment were combined in a video system
ve curves and digital figures were displayed on the
screen and included warning signals Sweeps for
nt curves are selected individually It is possible to
ast and slow changing parameters on the same
The system has great flexibility and signals from
ient from different manufacturers may be added

se of electronic equipment in monitoring the
on of the fetus has been established as an
tant tool in the obstetrical clinic An
tant advantage is the instant calculation of the
heart rate (FHR) based on a single beat to beat
al and the simultaneous recording of uterine
y Our present clinical use of these recordings
manly based on the relationship between
ets in FHR (decelerations and accelerations)
anges in the intrauterine pressure (IUP)

ronic monitoring in obstetrics is still a
ely young field and it is reasonable to expect
much more information can be obtained by
aning FHR and IUP with other parameters It
en suggested that there may be an important
ts between maternal respiration and FHR or
en IUP and fetal pH However with the
t type of equipment it is difficult to obtain
r other combinations of parameters in a
tably simple way both from an economic and
cal point of view

ning the planning phase of the new University
ment of Obstetrics and Gynecology at the
VCoun y Hospital a video display system has
developed and clinically evaluated This

system has great flexibility A combination of
parameters from different types of equipment is
easy and transmission of the information is
performed by a simple coaxial cable

SYSTEM DESIGN

The video picture is composed in the *video mixer*
which receives video signals from the five sources
(Fig 1)

(1) *Patient data* date and time are supplied by
the central hospital computer or by a TV camera
watching a digital clock and a sheet with patient
data and calibration lines

(2) *Dynamic signals* such as FHR IUP fetal and
maternal ECG respiration frequency are supplied
from bedside equipment and converted in an 8
channel analogue video converter (AVC 800)

(3) *Static signals* such as pulse rate temperature
instantaneous FHR may be supplied from bedside
equipment or from electronic equipment measuring
pH pO_2 or pCO_2 Static signals are converted in a
digital video converter (DVC 48) and displayed on
the screen next to the corresponding text

(4) The primary parameters from the delivery
room and the laboratory may pass through a
computer such as a Corometrics minicomputer
DPAC or the Hewlett Packard 5600 system and
these results can then be shown on the video
screen

(5) A camera placed in the delivery room makes it
possible to show a *picture of the mother or new born*
superimposed on the curves and figures or as
alternatives to these

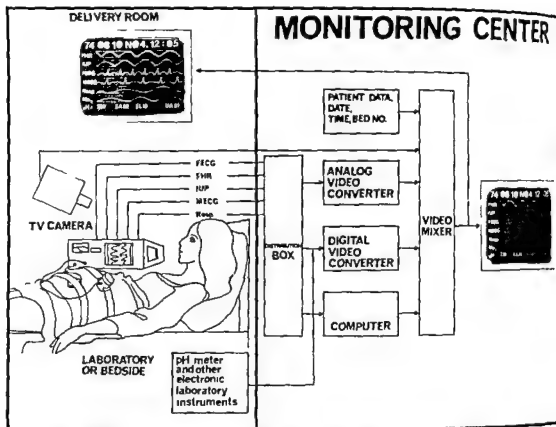


Fig 1 Block diagram of the system design. The primary signals from the electronic equipment at bedside and from the laboratory are converted to video signals by the analogue video converter and the digital video converter. These signals are mixed in the video mixer with patient data, time indication and results from the computer. A camera may add a picture of the mother or newborn. The video picture contains in this case Date, bed number

time, FHR and IUP as trend curves (previous 30 minutes), EECG and MECG (previous 5 seconds), trend curves showing maternal respiration and transcutaneous oxygen tension (previous 30 minutes), previous fetal pH and computer data (early deceleration area, late deceleration area and uterine activity). The video picture is shown on a monitor in the central monitoring centre as well as on a bedside

MONITORS AND VIDEO PICTURE

The video picture is shown on a monitor in the central monitoring centre and is transmitted by a coaxial cable to a medium size (12") monitor at the bedside. The video picture can also be transmitted to any place in the hospital by coaxial cables. Monitors are placed in conference rooms, lecture rooms and in the doctors' offices (Fig 2).

Sweep speeds between 2.5 seconds and 8 hours may be selected individually for each of the 8 channels. The immediate values are presented at the right edge of the monitor and shifted to the left at a rate determined by the sweep speed selected for the channel. This storing capacity is due to the 8 memories in the AVC 800.

It is possible to include alarm functions. If FHR passes upper or lower limits, the indication of FHR will flash on the screen. Such warning systems may be designed for excessive or strong uterine contractions or when the computer data indicates pathological conditions (e.g. decelerations, fetal abnormalities).

RECORDING

Recording of data is valuable for routine training and research. The video picture may be recorded in four different ways (Fig 2).

- (1) Polaroid photographs of the screen
- (2) Paper copies produced in a video hard copy unit

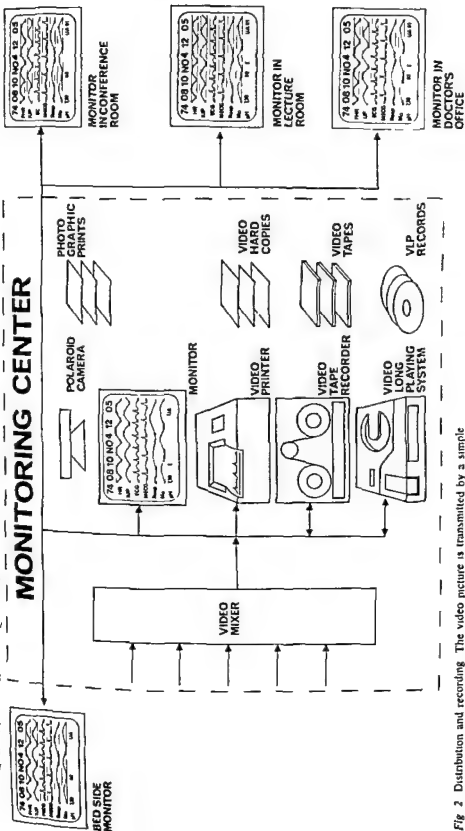


Fig 2 Distribution and recording. The video picture is transmitted by a simple coaxial cable and may be shown at any place in the hospital for example in conference rooms, lecture rooms and offices. The signals are recorded on Polaroid photographs, video hard copies, video tapes or video long playing records. When using the last two methods the signals can be demonstrated on monitors on later occasions for training or research.

(3) Recording on Video Tape Cassettes

(4) Recording of Video Discs on a Video Long Playing Recorder (not yet available)

The two last types of recording are preferable because they permit the data to be presented on monitors on later occasions as training or research material

COMMENT

The system has been evaluated clinically in the department during the past three months in daily routine use. It is easy to use after a minimum of instruction. The doctors, midwives and nurses have found the system valuable. The patients have reacted very positively because they were able to follow the recordings on the bed side video screen. This assured them that everything was under control.

The video system has a number of advantages compared with conventional monitoring systems using trend scopes or paper recorders. The primary parameters may come from equipment of different types. In our preliminary system assembly the fetal monitoring equipment was a Hewlett Packard cardiotocograph type 8020 A and the maternal parameters (MECG pulse respiration etc.) were measured at Philips Medical System modular units.

Analogue curves and digital figures are displayed

on the same screen and include warning signals in the form of flashing figures. As sweep switches, different curves are selected individually. It is possible to show fast and slow changing parameters on the same screen (FECG and FHR). Two features give a great flexibility and signals from equipment from different manufacturers may be added easily.

The recording methods are simple and need no documentation, training and research. The patient data and time unit ensures the identification.

The price of the system will not exceed that of a conventional monitoring system with a small number of read-out facilities. The digital converter may serve up to 8 beds with 6 sets of data for each patient. The video converter may be shared by two delivery rooms with 4 channels each. Further, the expensive memory trend scopes of the conventional systems can be replaced by simple video memory because of the memory capacity of the analog video converter.

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PREVENTION OF INTRAPERITONEAL ADHESIONS BY DEXTRAN HYDROCORTISONE AND CHYMOTRYPSIN¹

An Experimental Study

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The purpose of an experimental study in guinea pigs was to prevent adhesion formation in the intra-abdominal space by simultaneous intraperitoneal infusion of dextran, hydrocortisone, dextran and chymotrypsin. An examination made three weeks after the operation revealed a significant ($p < 0.001$) difference in favour of the dextran-chymotrypsin and dextran-chymotrypsin-hydrocortisone groups when compared with the control groups.

The occurrence of postoperative adhesions after abdominal and gynaecological laparotomy varies widely. Often the adhesions lead to complications such as secondary disease (18, 19). Corticoids, macro-molecular dextrans, hyaluronidase and fibrinolytic enzymes are known to inhibit adhesion formation. Some experimental (1, 2, 3, 4, 5, 6, 9, 10, 11, 12, 13, 15, 17).

In order to be able to minimize adhesion formation following operations for sterility, animal experiments were performed in an attempt to prevent intraperitoneal adhesions.

MATERIAL AND METHODS

Guinea pigs weighing 85-475 g were used. 201 animals were divided into experimental groups with 39-42 animals in each group. The following substances were used for intraperitoneal injections: Group 1: no injection (control group); Group 2: Isotonic NaCl 10 ml; Group 3: Dextran 10 ml; Group 4: Dextran 10 ml + Chymotrypsin 25 mg; Group 5: Dextran 10 ml + Chymotrypsin 25 mg + Hydrocortisone 10 mg.

Dextran = Rheomacrodex®; Lerais Hydrocortisone = Hydro-Aldeson®; Oy Organon Ab Chymotrypsin = Chymase®; Armour Pharmaceutical Company Ltd.

Operative technique

The animals were anesthetized with ether. Their skin was shaved, thoroughly cleaned, sprayed with a plastic skin dressing (Nobecutan® AB Bofors) and covered with a split towel. Sterile instruments were used, but strict aseptic conditions could not be maintained. The abdomen was opened with a short midline incision through which the caecum was pulled out. Trauma likely to lead to adhesion formation was caused by exerting intense pressure for 10 seconds with a Kelly's hemostat on the caecum at three sites about 5 mm apart. The caecum was then replaced in the abdominal cavity and the peritoneum and the muscular layer were closed with a continuous 00 catgut suture through the fascia. The outer muscle layer was closed immediately before the peritoneal suture was closed. The test substance was injected intraperitoneally. Care was taken to avoid spilling any liquid in the wound. The skin was closed with interrupted silk sutures. No antibiotics were administered post-operatively. The experimental animals were killed three weeks after the operation, their abdomens opened and the number and localization of adhesions recorded. In order to exclude the possibility of bias, the animals were examined without knowing the experimental group to which they belonged.

Numerical values were given to the different grades of adhesions as follows: no adhesions = 0; 1: very thin (hair line) adhesion; 2: thicker, distinct adhesion; 3: two very thin adhesions; 4: two thick or several adhesions.

For statistical analysis Student's *t* test was used.

RESULTS

The extent of adhesions in the 201 guinea pigs belonging to the five experimental groups is presented in Table I.

The number of adhesions in the dextran groups was significantly smaller ($p < 0.001$) than in the control group. Even the difference between the NaCl and the control group was in favour of the NaCl group ($p < 0.001$). The difference between the dex-

Table 1 Results of the degree of adhesions in the control and the experimental groups

0=no adhesions 1=very thin (hair line) adhesion 2=thicker distinct adhesion 3=two very thin adhesions thick or several adhesions

	No of animals	Degree of adhesions					Mean value	Standard deviation
		4	3	2	1	0		
Control groups	39	19	10	10	—	—	3.23	0.84
Isotonic NaCl	47	16	6	12	8	—	2.71	1.17
Dextran	41	5	5	6	8	16	1.38	1.29
Dextran + chymotrypsin	39	6	—	12	8	13	1.47	1.34
Dextran + chymotrypsin + hydrocortisone	40	1	7	8	4	20	1.18	1.74

trian groups and the NaCl group was statistically highly significant in favour of the dextran groups ($p < 0.001$). No significant differences between the dextran groups could be demonstrated. Nevertheless, in Group 5 in which chymotrypsin and hydrocortisone were used in addition to dextran, numerous adhesions were found in only one case. More than half of the animals were free from adhesions.

DISCUSSION

Our study definitely indicates the superiority of dextran over isotonic NaCl and absence of treatment in the prevention of adhesions, even though Aszodi et al. (1) could not demonstrate in their cat experiments that dextran was better than NaCl. On the other hand, Kapur et al. (9) obtained better results in preventing adhesions with dextran than in their control groups. Several investigators have additionally demonstrated the definite adhesion preventing effect of cortisone and hydrocortisone (4, 5, 11).

Swolin's studies (16) demonstrate conclusively that hydrocortisone administered in relatively massive doses in the investigations performed so far is effective in the prevention of intraperitoneal adhesions. Totterman & Arstila (20) have demonstrated that dextran definitely remains in the abdominal cavity for a longer period than physiologic saline and proposed that other substances infused into the abdominal cavity together with dextran would also remain there for a period longer than normal. Knightly et al. (12) used fibrinolysin and heparin to prevent adhesions and found that the best results were obtained by using the two substances simultaneously in the hydrotubation of

sterility patients. Grant & Robertson (17) used large amounts of hydrocortisone and chymotrypsin and obtained relatively good results in comparison with the control series. The simultaneous administration of three adhesion preventing substances is apparently exceptional but as our primary results in guinea pigs have been promising, they encourage us to continue our studies in clinical work both in the operative and postoperative therapy of sterility patients and in cases of ectopic pregnancy by infusing them intraperitoneally with this combination before the final peritoneal closure and later by daily hydrotubations.

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ANNOUNCEMENTS

An International Symposium on Hypertensive Disorders in Pregnancy will take place in Muenster Westf Federal Republic of Germany August 24-26 1976

The following topics will be discussed Morphology of the Kidney Pathophysiology Endocrine aspects Placenta and uterine blood flow Treatment aspects and nomenclature Free communications will be presented only in a limited number Languages English and German

For information write to the Congress Secretary Heinz D Böttcher M D Universitäts Frauenklinik Westring 11 4400 Munster/Westf Federal Republic of Germany

The 5th International Conference on Birth Defects will take place in Montreal on 21-27 August 1977 Organizing body National Foundation—March of Dimes

Further information can be had from the Secretariat c/o Holland Organizing Centre 16 Lange Voorhout The Hague the Netherlands

The International Society for Twin Studies announces the *Second International Congress on Twin Studies* to be held in Washington D C USA 29 August-1 September 1977

Further information can be obtained from Dr Gordon Allen Second International Congress on Twin Studies Clinical Center 2N757 National Institutes of Health Bethesda Maryland 20014 USA

German society for endocrinology Competitions for 1977

Award Schoeller Junkmann Award

Amount DM 15000 -

Donor Schering AG Berlin

Applicants must reside in Europe and not be older than 40 years

Subjects all fields of endocrinology (except diabetes mellitus)

Award Marius Tausk Career Development Award

Amount DM 15000 -

Donor Organon GmbH Munich

Applicants must reside in Europe and not be older than 33 years

Subjects clinical and clinical-experimental endocrinology (except diabetes mellitus)

Applicants are invited to submit two copies of previously unpublished papers in either German or English together with a short curriculum vitae and a description of the development of their scientific career with the President of the German Society for Endocrinology 1976/77 Prof Dr med Gerhard Bettendorf Universitäts Frauenklinik Martinstraße 51 2000 Hamburg later than October 15 1976

After receipt of the manuscript has been accepted by the German Society for Endocrinology the author is at liberty to have his paper published in a periodical

Detailed information concerning the awards can be obtained from the President of the Society The award will be presented at the 27th Symposium of the German Society of Endocrinology 1977

As a tribute to René Morard

Associate Professor in Hormonology at the Faculté de Médecine de Paris his friends have decided to award a prize of 125000 B F The best experimental work relating to the Initial Stages of the Cancer of the Cervix will be awarded with this prize Only experimental research on this topic will be considered. An award having already been awarded a prize will not be contemplated

At the latest on December 1 1976 manuscripts should be in the hands of Doctor R Volzke Gynécologie Obstétricale et Gynécologique Hôpital Universitaire Brugmann Place Van Gehuchten 4 1000 Brussels Belgium

THE EFFICACY OF MELPHALAN IN THE COMBINED CHEMOTHERAPY OF MALIGNANT TROPHOBLASTIC DISEASE

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Steadily progressive improvement in the diagnostic and therapeutic management of malignant trophoblastic disease has occurred over the last few decades. The most important diagnostic aid is the determination of serum HCG by sensitive radioimmunoassay which permits the detection of minute amounts of the hormone and thus discrimination between LH and HCG. Therapeutic advances include the use of combinations of different cytotoxic drugs which have increased the cure rate from approximately 40% to a range of 80-95% (1, 2, 3, 4). Malignant trophoblastic disease is rare in the Scandinavian countries which effectively limits opportunity to acquire experience within individual centers. Over a series of 13 cases has been collected at the Karolinska Hospital during the last 10 years. Patients who had resistance against methotrexate were successfully treated by a combination of an alkylating agent melphalan (Alkeran), actinomycin D and methotrexate. In 12 cases this modification of a triple therapy regimen proved out to be life saving.

MATERIAL AND METHODS

Cases consisted of 23 women, 11 of whom displayed metastasis at the time of initial discovery. Suspicion of molar disease was noted in 15 patients after incomplete evacuation, spontaneous abortion in three and pregnancy to another three. The diagnosis of malignant trophoblastic disease was based on a generally accepted parameter, i.e. serum HCG persisting without definite decline of the titer or rising during an observation period of 2 months following instrumental evacuation of a molar pregnancy or being secondary to spontaneous abortion or term delivery. Previous uterine hemorrhage, sub-involution of uterus, pathologic findings in uterine curettings indicating malignancy, pelvic arteriography, radiograph of the chest and scintigram of the liver and the brain were used as additional diagnostic criteria although the presence of any of them was not always required as proof of malignancy.

The regimen of the triple therapy course consisted of usually prescribed doses of methotrexate and actinomycin D given over a 5-day period. In addition melphalan

was administered by intravenous infusion for 6 hours on the first treatment day. Modification of the individual doses for subsequent courses was made after assessing any toxic symptoms or signs generally by a lowering of the melphalan dose. Details of the triple therapy regimen are shown in Table I.

RESULTS

The distribution of the case material between metastatic and non metastatic conditions as well as the overall therapeutic results appear in Table II.

One patient, 44 years of age, developed multiple metastases following a molar pregnancy. She had received various combinations of chemotherapy with agents including methotrexate, actinomycin D and chlorambucil. Methotrexate was also administered as a regional infusion and hysterectomy and nephrectomy were performed as part of the treatment. In spite of intense therapy the patient did not survive. Autopsy showed multiple metastases of choriocarcinoma in the lungs, brain, liver, kidney and vagina. A subsequent patient who developed resistance to methotrexate and actinomycin D and in whom appeared signs of increasing pulmonary metastases served as the first candidate to be given the new combination of chemotherapy which proved to be very effective.

Table I Triple therapy regimen applied in cases resistant against conventional methotrexate therapy

Methotrexate	12.5 mg \times 2 i.v. /day over 5 days
Actinomycin D	0.5 mg i.v. /day over 5 days
Melphalan	1.0 mg/kg in 500 ml glucose solution i.v. infusion during 6 hours on the first treatment day

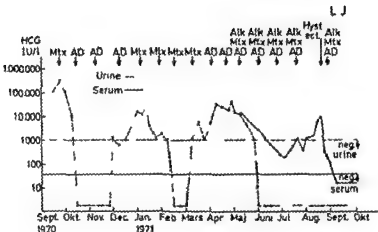


Fig 1 (Case 1) HCG levels during treatment. Mtx = methotrexate, Alk = actinomycin D, Alk = methylphenidate, Hyst. ect. = hysterectomy. Note that three therapy courses before hyst. ect. resulted in disappearance of the pulmonary metastases.

Case 1 (L J)

34 year old nullipara with three first trimester spontaneous abortions. After the last miscarriage vaginal bleeding continued which required repeat curettage 2 weeks later. Pathologic examination of the specimen revealed signs of malignancy and an X ray suggested pulmonary metastases. Subsequent developments are illustrated in detail in Fig 1. The initial methotrexate and actinomycin D courses resulted in HCG negative urine. Routine urine tests remained negative for a period of one month after which HCG reappeared. Actinomycin D alone had no effect and repeated methotrexate courses resulted only in brief periods of HCG negative urine. Ultimately complete drug resistance developed.

Triple therapy using an intravenous melphalan infusion over a 6-hour period on the first day of a 5-day course with methotrexate and actinomycin D resulted in a rapid response such that urinary HCG became negative and remained so for several months. It was noted also that the pulmonary metastases disappeared. However the serum HCG assay which had not been available earlier showed that there was still an active secretion of HCG in spite of four additional triple courses and pelvic examination revealed a persistently enlarged uterus. These findings in addition to a steadily increasing serum HCG level indicated the necessity of a hysterectomy. Surgery was performed 11 months after beginning methotrexate or three months following the first triple therapy course and resulted in a rapid decrease and disappearance of HCG. The pathology specimen demonstrated the presence of a viable choriocarcinoma within the uterine wall. Therapy was completed with a final triple therapy course and the patient has remained HCG negative for a 4 year period.

Case 2 (E L 25 years)

A positive pregnancy test was present 10 weeks following instrumental evacuation of hydatidiform mole showing signs of moderate trophoblastic proliferation. At pelvic examination there was a mass the size of a hen's egg to the left of the uterus and pelvic arteriography revealed a vascular tumor in the same area. Methotrexate 25 mg per day over 5 days and repeated one month later resulted in a rapid disappearance of urinary HCG but serum HCG remained at a level of 500-1000 IU/l and tended to increase

(Fig 2). Triple therapy with melphalan induced a continuous regression and serum became negative again one month following initiation of the course. A treatment course was given after which serum elevations remained negative. Four months later the patient became pregnant in spite of careful contraceptive measures. Legal abortion by vacuum aspiration showed a normal conceptus and the patient has been healthy since over an observation period of 3 years.

Case 3 (A L 79 years)

Four pregnancies of which two were benign hydatidiform moles and one was a spontaneous abortion. The last pregnancy went to term and the patient was delivered of a healthy boy (1971). In the spring of 1973 irregular vaginal bleeding began and diagnostic curettage was performed. No signs of pregnancy were noted in the specimen. In autumn of the same year vaginal bleeding recurred and another curettage was performed. Microscopic examination of the material showed chorionic tissue with a tendency towards proliferation. The urinary HCG level was 400 IU/l and an X ray film of the lungs showed the presence of a small suspicious density. The patient was referred from a community hospital to this department. On pelvic examination a marked enlargement of the uterus with only a slight enlargement of the uterine corpus was noted. The cervical canal was open for more than a finger and the internal surface of the uterus was covered with

Table II Malignant trophoblastic disease (L J) in the Hospital Stockholm 1966-1974

No. of patients	23
Non metastatic	11
Metastatic	12
Etiology	
Mole	16
Abortion	3
Term delivery	4
Result	
Alive	22
Dead	1

E L

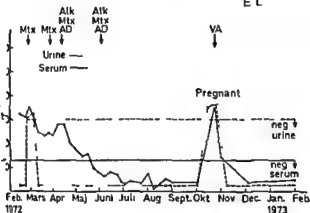


Fig 2 (Case 2) VA=vacuum aspiration. Note the tendency towards increasing serum HCG levels prior to the first triple course.

ayer of tumor. Serum HCG was high, namely 72,000 IU/l. The initial methotrexate course did not influence the serum HCG level, and triple therapy was started 4 weeks later (Fig 3). Serum HCG decreased markedly. The intra uterine tumor became soft and diminished in size. Hysterectomy was performed as a safety measure because of the patient's previous two molar pregnancies. Microscopic examination of the uterus showed no non-viable chorionocarcinoma-like tissue. X-ray examination of the lungs was negative ultimately, and the patient has been healthy with HCG negative serum over a follow-up period of almost one year.

(M.B. 21 years)

The patient had a legal abortion in 1972 and was delivered of a normal pregnancy in March 1974. She was delivered 10 weeks postpartum due to profuse vaginal bleeding which required instrumental evacuation (June 1974). The specimen revealed decidua and trophoblast with atypical proliferation. The serum HCG was not raised. Unfortunately, three weeks later the patient

developed acute lower abdominal pain. Upon admission she presented with signs of a massive intra-abdominal hemorrhage. A decision to perform a laparotomy was made, but an X-ray film of the lungs taken immediately prior to surgery showed widespread pulmonary opacities, strengthening the suspicion of metastatic trophoblastic disease and secondary perforation of the uterus. The abdomen contained at least 1000 ml of blood, and there was a small area of perforation on a walnut-sized soft tumor in the fundus of the uterus. In consideration of the patient's age, the tumor area was excised, and the uterine wall sutured together in two layers. The pathology showed a chorionocarcinoma. Serum HCG was 72,000 IU/l on this occasion.

Two courses of methotrexate later resulted in a significant decrease of the serum HCG levels, followed by a rise again (Fig 4). A triple therapy course induced a rapid fall in the titers, and the HCG test became negative three weeks later. A further triple therapy course was given one

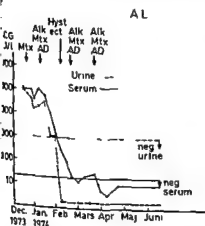


Fig 3 (Case 3) The high initial HCG levels did not respond to methotrexate alone. Note the rapid decrease in HCG following the first triple course.

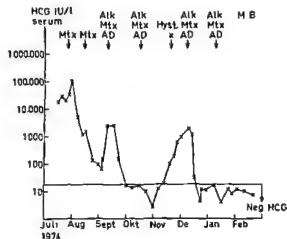


Fig 4 (Case 4) The patient developed resistance against methotrexate but responded favourably to repeated triple courses. The pathology specimen did not show any remnants of chronic tissue.

month later but following a period of three weeks HCG reappeared in serum and hysterectomy was performed (November 25 1974) since the uterus was slightly enlarged. However microscopic examination showed no remnants of the tumor in the myometrium. During the subsequent 14 days there was a further rise in the serum HCG levels and triple therapy was given again. This time there was a rapid fall in HCG and the test became negative. A fourth course was begun on January 7th 1975.

The widespread pulmonary metastases decreased rapidly following the first triple course. Doubtful pulmonary densities existed at the time of hysterectomy but the X ray films are perfectly normal at present. The patient has been HCG negative for 6 months.

The white cell and the platelet counts decreased in all patients following the triple courses and reached minimum levels at approximately 7-10 days following termination of the treatment cycle. The decline of the values did not reach alarming levels in any instance and there were no signs of persistent damage to the hematopoietic system.

DISCUSSION

Melphalan, an alkylating agent, has not been used to any extent previously in the treatment of malignant trophoblastic disease. The only report that we have found described the successful use of this compound in two cases of choriocarcinoma like tumor of the testis (8). Chlorambucil and cyclophosphamide represent the common alkylating agents which have been utilized in combined therapy regimens of this disease. The tumor suppressive effect of alkylating agents is rather similar for all the different compounds. However minor differences with regard to half life time, solubility and effects on the hematopoietic system do exist. It can not be claimed that melphalan has advantages over chlorambucil and cyclophosphamide either on the basis of theoretical considerations or clinical experience. In spite of this it seems obvious that melphalan has a marked tumor suppressive effect in patients resistant to methotrexate. The dose utilized certainly was well tolerated.

In accordance with earlier experience the present series illustrates that hysterectomy need not be a

primary step in the treatment of malignant trophoblastic disease. Among our 11 patients hysterectomy could be maintained in 19 and on the basis of pathology reports it seems that hysterectomy was unnecessary in two of the three patients. The results also illustrate that surgery in spite of the efficacy of modern chemotherapy may still play an important role in the management of gestational trophoblastic neoplasms.

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CASF REPORTS

CHORIOANGIOMA WITH HYDRAMNIOS AND INTRA UTERINE FETAL DEATH

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Chorioangiomas are benign placental tumours from fetal vessels that occur with a frequency of about 1%. Often they are associated with hydrops and intra-uterine fetal death. Based on a case of chorioangiomas with a fatal course for the fetus the pathogenesis of hydrops is discussed. Future development in ultrasound technology facilitate antenatal diagnosis.

Chorioangiomas are benign placental tumours derived from the fetal vessels. They are considered hamartomas (3, 8). In larger series they occur with an approximate frequency of 1% (10, 13). They measure a few centimetres across and are situated within the placental substance. Large chorioangiomas (1, 4, 8) are rare, whereas the smaller ones are relatively often multiple (2).

Chorioangiomas are frequently associated with hydrops (33% of Sidall's (20) 110 cases, 25% of Knøth's (7) 163 cases). Intra-uterine fetal death is very high (35% of Strakosch's (11) 100 cases).

This paper illustrates a case of multiple chorioangiomas associated with fast developing hydrops and intra-uterine fetal death. Based on an analysis of the topography of the tumour, certain aspects of the pathogenesis of hydrops and the possibility of antenatal diagnosis are discussed.

CASE HISTORY

A 32-year-old primigravida was admitted to the Department of Obstetrics and Gynecology on December 23, 1975, three weeks prior to the estimated date of delivery. She had a rapidly increasing hydrops. Her weight was 70 kg (normal weight 50 kg) and the abdominal girth was 100 cm. The single fetus (X rays) was in a cephalic

position with a normal fetal heart rate. The patient, who showed no signs of preeclampsia or diabetes mellitus, was not in labour.

As the hormone analysis on a 24 hr. urine sample would be delayed due to the Christmas holidays, the patient was controlled daily with the cardiotocograph. No signs of fetal distress were observed during the first 3 days. On December 27, no fetal heart beats could be observed, and amniocentesis showed meconium stained liquor amnii. Emergency ultrasound scanning showed no fetal heart action, polyhydramnios and a large placenta reaching from 10 cm above the symphysis to the uterine fundus (Fig. 1). The delayed urine analysis showed oestriol 4 mg/24 hr. Plasma chorionosomatotrophic hormone (HPL) was 16.4 µg/ml. Labour was induced and the patient delivered a dead girl (3250 g, 50 cm). At autopsy no abnormalities were found. The heart weight was normal (16 g).

The placenta was large and oedematous, measuring 21×20×4 cm, weighing 1150 g. The abundant membranes were discolored by meconium. The cord, which entered 4 cm from the margin, measured 32 cm and contained three vessels. Numerous parallel sections through the placenta revealed multiple, typical chorioangiomas, measuring from 1×1×1 cm to 3×4×4 cm. The tumours were not spe-

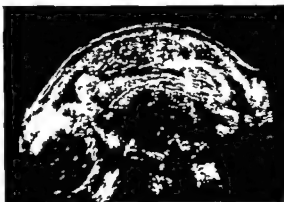


Fig. 1



Fig. 2

cifically concentrated around the entry of the cord (Fig. 2). Microscopy (Fig. 3) confirmed the diagnosis showing large trophoblast lined nodules packed with thin walled often dilated capillaries. Many single terminal villi with numerous vessels were interspersed among normal villi. Minor parts of the nodules showed hemorrhagic infarction.

DISCUSSION

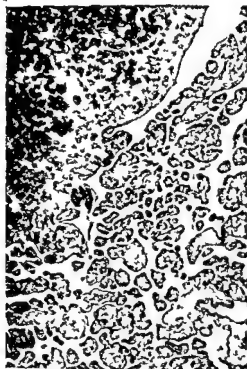
Studies on the mechanism of amniotic fluid formation (5) have shown that the exchange of liquor amnii in the so called three-compartment system (mother-fetus-liquor amnii) under normal conditions is by far largest between mother and fetus through the placenta. With the rapid exchange rate of amniotic fluid (approximately one third of the total volume per hour (12)) even the slightest imbalance (few ml) in the normal exchange could result in accumulation of liquor due to a decrease in removal of or an increase in production of amniotic fluid.

It must be stated however that the mechanism of the development of hydramnios is not settled even though it is clearly related to the chorioangiomas. Kühnel (7) thought it to be due to venous obstruction within the placenta whereas Benson & Joseph (1) argued that the tumour growth increased the fetal surface of the amnion thereby increasing the production of liquor amnii. Klaffen (6)

mentioned that hydramnios was more frequent the closer the tumour was situated to the entry of the umbilical cord thus causing strangulation of the large umbilical vessels. Several authors (1, 7, 8) consider the chorioangioma a "dead space" which causes recirculation to the fetus of blood with low content of oxygen and other essential metabolites and still containing waste products.

In the present case there was no close relationship to the entry of the umbilical cord (Fig. 2). Based on a topographic examination the total amount of solid well defined nodules was 10% of the placental volume. Microscopy however revealed that larger parts of the placental volume between the tumours consisted of a mixture of normal villi and small chorioangiomas which of necessity partly preserved villous configuration. If the microscopic findings are true for the placental whole then the total mass of tumours must be reduced to half the placental volume thereby explaining the large size. There was no general decrease in placental function since the HPL was relatively increased.

The dead space theory seems to be a logical explanation for fetal distress where no obstruction of the large umbilical vessels could be demonstrated. A change in normal circulatory conditions leading to excess strain on the fetal heart rate



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It in intra uterine fetal death. The heart will maintain the normal blood volume of 400-500 ml by increasing its capacity. The relative size of the infant heart could be explained by the rapidly developing hydramnios.

Hydramnios is not caused by anencephaly or immunisation, twin pregnancy or diabetes. If then multiple or large solitary chorioangioma should be suspected. At the present time ultrasound scanning of the placenta will not reveal it but the placental size and position. However, Knott (1975) (personal communication) claims that ultrasound scanning may in the future present a better picture of the placental interior thus making an antenatal diagnosis of chorioangioma possible.

BOOKS RECEIVED

Psychological Aspects of a First Pregnancy and Early Postnatal Adaption by P. M. Shereshefsky and L. J. Yarrow. North Holland Publishing Company, Amsterdam, 1973. 373 pp. Price Dfl 38.00, US\$ 14.60.

This highly valuable book is unique in its disposition, being intended for gynecologists, general practitioners, midwives, psychologists but also for a more heterogeneous public. The book gives a profound analysis on various

psychological factors having influence on the expectancies of pregnancy, on delivery and on the postnatal period as well. Furthermore, the book evaluates not only the role of the mother in all these events but also the father's. Considering the fact that the methodology part of the work is also of excellent quality, this book constitutes an important achievement in the modern preventive obstetrics.

45 XO TURNER'S SYNDROME WITHOUT EVIDENCE OF MOSAICISM IN A PATIENT WITH TWO PREGNANCIES

John Philip and Vagn Sele

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Abstract A case of Turner's syndrome (45,XO) with two pregnancies is reported. There was no evidence of mosaicism after chromosomal investigation of 5 different tissues (arteries, blood, skin, uterus). Pregnancy occurred in cases after withdrawal of substitutional hormone therapy. The first pregnancy ended with abortion of a retarded hydrocephalic female fetus. Chromosome studies could not be carried out. The second pregnancy ended with delivery by caesarean section of a normal boy. Prenatal chromosome analysis was not carried out for ethical reasons.

An unsatisfactory definition of Turner's syndrome is yet available. The definition should include an aetiological explanation. Ferguson-Smith (3) has put forward the hypothesis that monosomy of the short arm of the X chromosome is the decisive factor in the origin of the syndrome of short stature, congenital malformations. There is no agreement, however, on the number and character of the malformations or the state of the gonads that is to be found.

For these reasons and also because of its rarity it is of interest to publish a third case of so-called Turner's syndrome in which pregnancy occurred. Furthermore the case is of interest because chromosome complement of several tissues including both ovaries could be investigated and because the patient got pregnant twice. A more detailed report of the hormonal investigation will be published later.

CASE HISTORY

The patient is 147 cm high. She has slight cubitus valgus, no pterygium colli or other malformations have been found. She is married.

She was infantile without secondary sex characteristics when first seen 19 years ago, but after being treated from her 15th year with synthetic oestrogens (and later genuine oestrogens) she developed sex characteristics and has had regular vaginal bleeding. When 30 years old she suddenly stopped her hormone intake. 3 months later she was found to be pregnant. The diagnosis was established by means of ultrasound scanning (Fig. 1) and elevated excretion of oestriol (maximum 2.5 mg/24 hrs).

A spontaneous abortion occurred in a hospital in another part of the country at about 26 weeks of gestation. The fetus was of female sex, stillborn, macerated and hydrocephalic. It weighed 600 g and measured 34 cm. No tissue was obtained for chromosome analysis.

An exploratory laparotomy was carried out about a year later. The uterus was found to be normal. On the right side the gonad was represented by a streak, 2.5 × 0.5 × 0.5 cm. In direct connection with this streak a cyst like structure 1.5 cm in diameter was found. On the left side a very small but normal looking ovary was present though without visible follicles or corpora lutea (Fig. 2).

Biopsies from the ovaries showed normal ovarian stroma with very few follicles on both sides. Two primary follicles but no antral and Graafian follicles or corpora lutea were found on step sections. An atretic follicle was seen in the biopsy from the left ovary (Fig. 3).



Fig. 1 Ultrasonography of uterus in about 20th week of the patient's 1st pregnancy showing the fetal head.



Fig. 2 Photograph of the uterus and ovaries during operation. Left: the small but otherwise almost normal ovary is seen. Right: the cyst is shown in close connection with the streak.

As the patient hoped for another pregnancy stimulation therapy was carried out with large doses of gonadotrophic hormones: stimulation had no effect and no elevation of oestrogens in the urine was found. Vaginal bleeding recommenced following the stimulation; however, she started again on oestrogen therapy but stopped in December 1973. In February she took a few tablets but stopped again and had a vaginal bleeding. In April 1974 she was again pregnant. The uterus was enlarged quantitatively.

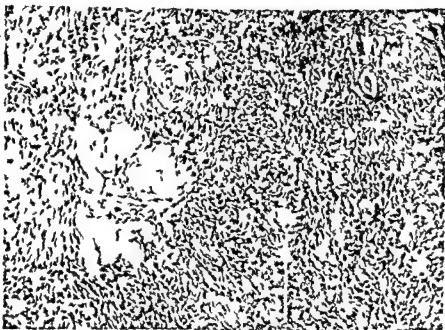


Fig. 3 Biopsy from the ovary showing two small follicles and an atretic follicle (H.E. x152).

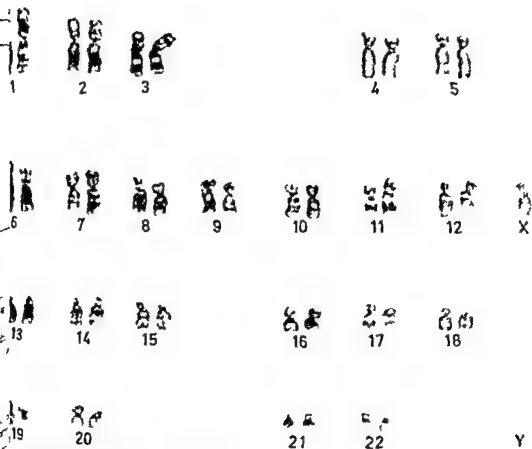
Table 1 Results of chromosome analysis

Tissue	43	43	44	45	46	47	48	49	50
Patient 45 XO									
Peripheral blood (two cultures)		1	1	4	1	1	1	1	1
Skin		1	1	1	1	1	1	1	1
Uterus									
Right ovary		1	1	1	1	1	1	1	1
Left ovary									
Total		1	2	6	7	3	3	3	3
Child (46 XY)									
Amniotic cells (7th month)									
Blood (after delivery)									

HCG showed 3700 IU/l. Ultrasound scanning definitely established the diagnosis by showing a normal pelvic configuration. She was delivered by caesarean section of a normal boy weighing 2900 g after about 9 months' pregnancy during which she was admitted to the hospital and closely monitored by ultrasound, urinary oestrogen excretion and serum HPL levels. Apart from the minor complications occurred, Alpha-fetoprotein in the amniotic fluid was normal.

Chromosome studies

The results of the chromosome studies are seen in Table 1. Cells from the patient's peripheral blood, skin and both ovaries were investigated. Blood was examined twice. A total of 215 mitoses were counted. 145 were 45 XO complement. Only three of the normal 46 chromosomes. One of these was from the mother and had a 45 XO + E complement. The other two



Karyotype from right ovary Trypsin-Giemsa showing 45 chromosomes. One sex chromosome missing.

blood and could not be analysed in detail (very old preparation). The nine others had hypomodal numbers. A smear showed no X or Y chromatin. Study of the karyotype of the patient did not reveal evidence of the presence of translocations including the missing X chromosome (Fig. 4). The chromosome complement of the fetus was studied in amniotic cells in the 7th month of the pregnancy because of hydramnios and too late for interference (abortion) and on blood after delivery and a normal 46 XY chromosome constitution was found in both tissues.

DISCUSSION

was decided not to offer the patient an amniotic cells in the second pregnancy because of the serious ethical problems involved in case of finding a 45 XO chromosome complement in the fetus. As she developed hydramnios after 7 months around scanning and amniocentesis was carried

out with a view to examining alpha-fetoprotein concentrations in amniotic fluid. At the same time a chromosome analysis was carried out because it was considered that a diagnosis of 45 XO in the fetus at that stage of pregnancy would not by itself indicate termination of the pregnancy. She was delivered by caesarian section of a normal boy (46 XY) without congenital malformations.

Since the end of the pregnancy the oestrogen excretion has been very low. More than 7 months after delivery oestrogens in urine are at 4-6 µg/l. A more detailed report of the hormonal status will be published elsewhere.

It is well known that a missing X chromosome does not interfere with the presence of oocytes in the ovary as it has been shown that fetuses with 45 XO complement do have normal ovaries containing germ cells in early fetal life (2). The disappearance rate for germ cells must therefore be af

ected by the missing X or missing part of the short arm of the X chromosome. The reason for this is unknown. It is possible that some quantitative effect is acting and therefore rare cases such as the one published here are only representing an extreme variation of the normal picture of Turner's syndrome. Mosaicism is another possible explanation. Although this possibility is always present, no evidence of mosaicism was detected by a study of five different tissues.

Two other cases of Turner's syndrome in which pregnancies proceeded to term are reported in the literature (1-4). The present case is the only one where two pregnancies have occurred and where mosaicism is deemed highly improbable. Several reports may be found in the literature of Turner patients having shown signs of some ovarian function (5) and therefore it does not seem reasonable to include streak ovaries in the definition of Turner's syndrome.

ACKNOWLEDGEMENT

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A PROSPECTIVE STUDY OF DRUGS AND PREGNANCY

4 Miscellaneous drugs

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Abstract The results are presented of a prospective study of drug use during pregnancy involving antibiotics, analgesic drugs and iron and vitamin preparations. The study was conducted in Malmö between 1963 and 1965. No favourable effect of the use of antibiotics, mainly penicillin and sulphonamides, could be demonstrated among 15 women who had an infant with hypospadias. No one had used penicillin during the first trimester, but this may well be coincidental. Analgesic drug use shows a similarity which resembles that previously described for psychopharmaca. No effect on the malformation rate or on survival could be found. A possible lengthening of mean duration of pregnancy occurred after the use of analgesic drugs during the 2nd or 3rd trimesters. Women who are going to have a dead or malformed infant use iron and/or vitamin preparations less often during late pregnancy than women who prove to have a normal infant. When such drugs were used, the percentage of pregnancies ending in birth before the 38th week is reduced and the birth weight among term babies is higher. The associations between pregnancy outcome and the use of iron and vitamin preparations is probably indirect, due to other factors associated with drug use.

This paper reports data from a prospective study of drug use during pregnancy carried out in Malmö during 1963-1965. In three earlier reports the use of psychopharmaca, antiemetics and endocrine preparations were described. The present paper summarizes data on miscellaneous drugs: antibiotics, analgesics and iron or vitamin preparations. Antibiotics have been studied for a teratogenic effect (cf. 5) and early claims exist for such an effect. The harmful effect of tetracyclines on dental development has been demonstrated (15) and the possibility that they can cause true malformations is still open to debate (14). Some studies (e.g. 13) suggested that the use of analgesic drugs during

early pregnancy may harm the embryo, but others (4) could not verify this.

MATERIAL AND METHODS

The study is based on information collected prospectively from 6376 women. The first paper of this series (8) describes the details of the study.

RESULTS

1 Antibiotics

Fig. 1 shows the overall use of antibiotics during the different months of pregnancy and according to pregnancy outcome: miscarriage or birth of a living infant. Among a further 123 pregnancies ending in

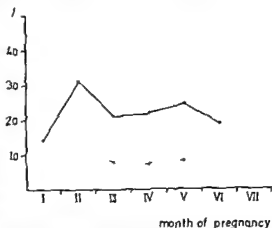


Fig. 1 Percentage of women using antibiotics during different months of gestation — miscarriage - - - living infant

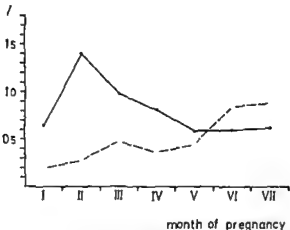


Fig 2 Incidence of recorded infectious diseases with fever (—) and of urinary tract infections (---) during different months

an induced abortion antibiotics were used by a maximum of five women each month of pregnancy—a number too small to make comparisons meaningful. Among 68 pregnancies ending with a stillbirth only two women used antibiotics.

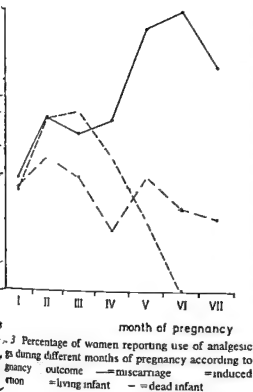
Fig 1 shows that women who will later miscarry show a markedly higher drug use than women who will later have a living infant. For both categories maximum antibiotic use occurred during the second month of pregnancy. Then 72 women who later had a living infant used antibiotics out of a total of

5684—among 417 women who later had a miscarriage 13 used antibiotics ($\chi^2=9.69$ at 1 d.f., $0.01>P>0.001$). During the first month of pregnancy—i.e. before the first missed period—the miscarriage group also shows more than double the incidence of antibiotic use compared with the group with live births but statistical significance is not reached ($\chi^2=3.5$ after normal transformation at 1 d.f., $0.1>P>0.05$). The reason for the peak incidence of antibiotic use during the second month is explained from Fig 2 showing the incidence of infection with fever—mainly upper respiratory tract infections—and of urinary tract infections in the same series. The former graph has a definite peak during the second month and then tapers off to a steady state after the fourth month level with that of the first month. Urinary tract infections increase in incidence—or detection—during the whole of pregnancy. The main drugs used for these diseases are penicillin and sulphonamides. Table 1 shows the use of these two drugs during the first trimester and the outcome of the pregnancy. There are no indications that either of the drugs is harmful to the embryo but so few cases are included that a marked teratogenic effect cannot be excluded from these data. One interesting point is that among pregnancies ending with the birth of an infant with hypospadias three had used penicillin during the first trimester.

Table I Number of women using penicillin or sulphonamides during the first trimester

Infants with major malformations and matched controls: infants without malformations but dead within one year and matched controls. Expected numbers calculated from the incidence of antibiotic users in the total group of women giving birth to non-malformed infants (128/5 007) or from all controls.

Infant group	Total number	Number using penicillin	Expected number from total mat	Number using sulfa	Expected number from total mat
Major malformation	194	5	5.0	4	6.9
Controls to major malformation	194	7	5.0	3	6.9
Dead but not malformed	93	4	2.4	2	3.3
Controls to dead but not malformed	93	6	2.4	2	3.3
Dead and/or major malformation	287	9	7.3	6	10.3
All controls	287	13	7.3	5	10.3
Specific malformation types					
C.N.S. malformation	11	0	0.5	0	0.7
Cleft lip and/or palate	10	0	0.5	0	0.7
Cong. heart malformation ± other malformation	35	0	1.6	0	0.6
Hypospadias	15	3	0.7	1	0.3
Cong. dysplasia of hip	37	1	1.7	0	0.6
Multiple malformation	10	1	0.5	1	0.7
Mb Down	10	0	0.5	0	0.7



2 Analgesic drugs

Analgesic drugs—mainly preparations containing acetylsalicylic acid—are used freely during pregnancy as is seen in Fig 3. Already during the first gestational month—before the first missed period—women who will later have a living child have a lower consumption than women who will have a spontaneous abortion, a stillbirth or an induced abortion. χ^2 analysis for heterogeneity between these groups gives $\chi^2=10.4$ at 3 d.f. $0.02 > P > 0.01$. The small number of women who end with a stillbirth (68) makes it impossible to decide if they belong to the same group as women who have a living child or to the group of women who will miscarry. In the subsequent months of gestation the graph of analgesic takers who will have a live birth and those who will have a stillbirth follow each other closely—only during the fourth month is there a difference but this is not statistically significant ($\chi^2=2.5$ at 1 d.f. N.S.). The graph based on women who will later have a miscarriage shows a marked rise compared with that based on women who will have a stillbirth. The latter shows a maximum in the second month but the former continues to rise. The graph

based on women who will have an induced abortion follows that for women who will have a spontaneous abortion during the first three months but then turns downwards abruptly.

As is visible from Fig 4 there is no clear-cut difference in analgesic use among women who will have infants of different types: normal infants in infants with minor malformation or with major malformations. There is a tendency for the graph based on women who will have infants with minor malformations to be slightly elevated especially during the third and fourth month. This difference is however of minimal significance (χ^2 for heterogeneity between these three groups during the fourth month gives $\chi^2=6.1$ at 2 d.f. $P \sim 0.05$). A slightly increased incidence of analgesic use among women with an unwanted pregnancy is found—32.4% against 29.8% among women with a wanted pregnancy—but this is not statistically significant ($\chi^2=2.89$ N.S.). Women married at L.M.P. use analgesics slightly more often than women not married at L.M.P. (32.1 against 29.8% $\chi^2=3.4$ N.S.). These differences resemble but are not as strong as those found for psychopharmaca. The mean age of women using analgesics is somewhat elevated (26.83 years against 26.21 for non-users $t=2.95$ $0.01 > P > 0.001$) again a trend similar to that found for psychopharmaca.

Table II compares the use of acetylsalicylic acid (with the possible addition of caffeine or codeine

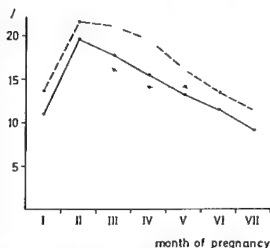


Fig 4 Percentage of women reporting use of analgesic drugs during different months of pregnancy. Comparison between normal infants (—) infants with minor (---) or major (....) malformations

Table II Number of women using salicylates during the first trimester

Infants with major malformations and matched controls
Infants without major malformations but dead within one year with matched controls

Infant group	Total number	No using salicylates	
Major malformation	194	34	
Controls to major malformation	194	33	
Dead but not malformed	93	12	
Controls to dead but not malformed	93	23	
Dead and/or malformed	287	46	
All controls	287	56	
			Expected no from all controls
<i>Specific malformation types</i>			
C N S malformation	11	2	2
Cleft lip and/or palate	10	1	2
Cong heart malformation±other	35	7	7
Hypospadias	15	2	3
Cong dysplasia of hip	37	7	7
Multiple malformation	10	0	2
Mb Down	10	2	2

and the outcome of pregnancy. No signs of embryotoxic or teratogenic effect can be found—there is even a lower incidence of drug use in the group of women who will have an infant dead within

one year than in the other groups. According to Table III there is no definite difference in the length of pregnancy if the woman used a salicylate during any trimester or not. There is, however, a trend in the group that only used such drugs during the 2nd–3rd trimester when compared with the group that did not use analgesic drugs at all, namely a surplus of prolonged and a slight deficiency of premature pregnancies with a male infant. When these two groups are compared with a χ^2 -test for trend (12) a possibly significant difference is found $\chi^2=4.9$ at 1 d.f. $0.05>P>0.01$.

Table IV shows that there is no difference with respect to birth weight.

A special study was performed on women who had used phenylbutazone or oxyphenbutazone. In women were found that had used one of these drugs during the first trimester. Among these one had a miscarriage, six had infants with minor malformations, and one had an infant with a major malformation.

3 Iron and vitamin preparations

The use of iron and/or vitamin preparations is shown in Fig. 5. Women who will have an induced abortion show a markedly lower usage than the others. Also women who will have a miscarriage show a reduced intake of such drugs—especially during the later part of the pregnancy period. Interestingly enough there is also from the 6th month onward a reduced intake in women who will prove to have a dead child. A very faint similar

Table III Duration of pregnancy when analgesic drugs were used during the first trimester during the second or third but not the first trimester and when such drugs were not used at all

Expected numbers (calculated for each sex separately) given within brackets for pregnancies shorter or longer than 38 weeks

	Total number	Duration of pregnancy			χ^2 (4 d.f.)
		Less than 38 weeks	38-42 weeks	More than 42 weeks	
<i>Boys</i>					
Drug first trimester	654	48 (50)	579	27 (27)	6.71 N.S.
Drug 2nd-3rd trimester	645	43 (49)	566	36 (27)	
No drug	1 090	90 (83)	963	37 (46)	
<i>Girls</i>					
Drug first trimester	696	54 (54)	618	24 (21)	0.48 N.S.
Drug 2nd-3rd trimester	644	46 (50)	578	20 (21)	
No drug	1 037	83 (80)	971	33 (34)	

χ^2 between drug groups within sexes = 6.69 N.S. at 8 d.f.

Table IV Birth weight in non malformed infants when the woman had or had not used analgesic drugs during the first trimester during the second or third but not the first trimester and not at all

	Total number	Birth weight kg						χ^2 (10 d f)
		<2.5	2.5-3	3-3.5	3.5-4	4-4.5	>4.5	
1st trimester	654	19	78	201	228	106	22	8.36 N.S.
2nd-3rd trimester	645	22	60	214	230	100	19	
Drug	1 090	48	111	365	384	155	27	
1st trimester	696	33	105	264	270	67	12	3.09 N.S.
2nd-3rd trimester	644	29	100	253	198	50	14	
Drug	1 037	42	173	411	313	79	19	

Between drug groups within sexes = 11.45 at 20 d.f. N.S.

is visible also for women who will later on have a child with a major malformation (Fig. 6) and these two groups are added (perinatal pathology) and compared with the group of women who have a living normal child: the difference is not significant. During the seventh month 59% of the first women and 67% of the latter use iron and/or vitamin preparations ($\chi^2 = 5.5$, $0.02 > P > 0.01$). The use of iron and vitamin preparations during pregnancy is thus similar irrespective of the time of the pregnancy—the only exception being induced abortions. The mean age of women using such drugs is slightly higher than the mean age of women not using these drugs, but the difference is not significant (26.6 and 26.3 years, respectively, $t = 1.5$). The acceptability of the pregnancy and the status of the women at L.M.P. influence the use of these drugs markedly. Among women with a desired pregnancy 38.7% use these drugs during the first trimester against 28.0% among women with an unwanted pregnancy ($\chi^2 = 44.3$, $P < 0.001$). Corresponding figures for women married at L.M.P. and not married at L.M.P. are 40.5 and 16.7%, respectively ($\chi^2 = 52.5$, $P < 0.001$). After the first trimester virtually all women receiving a wanted pregnancy have used iron and/or vitamins, but only 74% of women who have an unwanted pregnancy—the same difference is seen among women married at L.M.P. (near 100%) and not married at L.M.P. (87%). The mean age of women using these drugs after the first trimester is 26.39 years—nearly identical with that of all women included, 26.37 years.

Table V compares the duration of pregnancy

when iron vitamin preparations had been used (any trimester) or had not been used. There is a highly significant difference between the two groups, mainly due to differences in the incidence of pregnancies lasting less than 38 weeks. In Fig. 7 this group is broken down further. It appears that the presence or absence of other drugs besides the iron vitamin preparations has little significance for the incidence of pregnancies of less than 38 weeks.

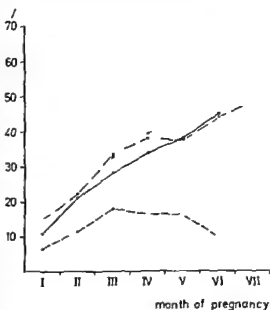


Fig. 5 Percentage of women using iron and/or vitamin preparations during different months of pregnancy according to pregnancy outcome: — miscarriage, - - induced abortion, . . living infant, - . dead infant.

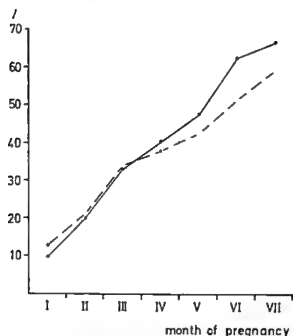


Fig 6 Percentage of women using iron and/or vitamin preparations during different months of pregnancy. Comparison between living normal infants (—) infants with major malformations (---) and with infants with perinatal pathology (i.e. stillbirth and/or major malformation) (---)

In Table VI birth weights are compared for normal infants born to women who used or did not use iron vitamin preparations and with a gestational length of 38–42 weeks. For boys a significant difference is found with an excess of low birth weight infants in the group that did not take iron vitamins during pregnancy and a similar trend is seen also for girls. Fig 8 illustrates this phenomenon.

Table V Duration of pregnancy when iron vitamin preparations were or were not used during pregnancy (any trimester)

Expected numbers (calculated for each sex separately) given within brackets for pregnancies shorter or longer than 38 weeks

		Duration of pregnancy			
	Total number	Less than 38 weeks	38-42 weeks	More than 42 weeks	χ^2 (2 d f)
<i>Boys</i>					
Drug	1 878	123 (142)	1 674	81 (79)	12.2
No drug	511	58 (39)	434	17 (21)	
<i>Girls</i>					
Drug	1 935	109 (131)	1 761	65 (63)	21.6
No drug	442	52 (30)	378	12 (14)	

0.01 > P > 0.001 * P < 0.001

DISCUSSION

No definite teratogenic effect has been demonstrated with penicillin or sulphonamide which agrees with the general view on the subject (d. 18). We have demonstrated however that women who will later have a miscarriage use antibiotics or sulphonamides more frequently than women whose pregnancies will go to term with certainty during the second gestational month but probably also during the first. A reasonable explanation would be that these drugs have been prescribed for infectious diseases which themselves may cause miscarriage. The high incidence of infectious diseases—and therefore also of antibiotic use—during the second pregnancy month may be related to the high HCG levels during that part of the pregnancy (?) as it has been claimed that HCG during pregnancy can have an immunological function and reduce maternal defence against infection.

Relatively few women used penicillin during the first trimester. Among 5 002 women that gave birth to an infant 128 report the use of penicillin—these had infants with major malformations which is just the expected number. The recorded figure could however be a random underestimation of the true number of 10 ($P=0.05$) and therefore the recorded lack of teratogenicity could hide a deviation in malformation incidence. There are no facts indicating that this would be the case—the only observation is perhaps that three of the five infants with hypospadias. It is hardly likely that penicillin can cause hypospadias but it may be used to treat a relevant infection. This is mere speculation however.

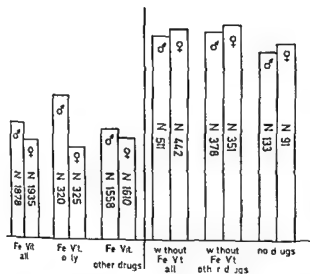


Fig 7 Percentage of infants born before the 38th gestational week according to drug use during pregnancy (any trimester) Fe Vit=iron and/or vitamin preparations N gives number of women in each group

The pattern of analgesic use apparent in this study shows trends resembling those found for isotropic drugs (8). There is already a higher use of analgesics during the first gestational trimester among women who will later on have a miscarriage. It is common experience that analgesics are often used instead of psychopharmaca by women. In women who will have a miscarriage there is a steady increase in use of analgesics especially during months five and seven. This may follow vaginal bleeding and other complications related to the abortion.

There is little difference in the use of analgesics in women who will have a malformed child and in women who will have a normal child, but there is a decrease in analgesic use during the third

month among women who will later on have a child with minor malformations—but not with major malformations. Richards (13) stated that a significantly increased use of analgesics was found during the first trimester among women who will have a malformed child. However, his data was obtained by retrospective questioning. So for instance, strong and significant correlation between birth of malformed children and unbalanced or doubtful diet was found. Such findings probably suggest bias in the collection of data and not real differences. Crombie et al (4) on the contrary found a slightly lower consumption of analgesics among women who had a malformed child than among women who had a normal child. Interestingly enough, Nelson & Forfar (12) also found an increased use of analgesics

Table VI Birth weight in non malformed infants when the woman had or had not taken iron vitamin preparations during pregnancy (any trimester)
Gestational length 38–42 weeks

	Total number	Birth weight kg						χ^2 (5 d f)
		<2.5	2.5–3	3–3.5	3.5–4	4–4.5	>4.5	
Drug	1674	25	139	565	612	275	58	13.5 ()
	434	14	40	138	176	59	7	
No drug	1761	36	258	709	562	159	37	5.8 N.S.
	378	13	56	149	129	25	6	

Between groups within sexes = 19.3 at 10 d f 0.05 > P > 0.025
0.01 > P > 0.01

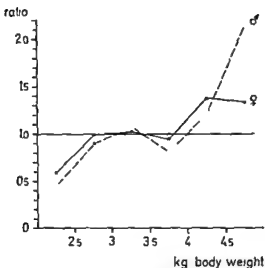


Fig 8 Ratio showing the percentage of infants within each weight class whose mothers used iron and/or vitamin preparations divided with the percentage of infants whose mothers did not use such drugs. Ratios below 1 thus indicate that the former group is underrepresented ratios above 1 that the latter group is so —=boys —=girls

among women who later on had a malformed infant. The studies of Richards and Nelson & Forfar are both retrospective but the present study like that of Crombie et al is prospective. It is very likely that the different results concerning the use of analgesics are mainly due to the different modes of collection of data and the bias inherent in retrospective studies. Villumsen's (17) prospective study showed a slight but not statistically significant increase in use of analgesics among women who had a malformed child. There is an indication in our data that the use of analgesic drugs can influence the duration of pregnancy. Women who later on had a male child had on the average a longer pregnancy when an analgesic drug had been used than when this was not the case. The difference was not very strong and could be the effect of many different causes—one possibility is through the inhibition of prostaglandin synthesis and release (10, 16).

Perhaps the most marked influence of drug use recorded by us is that of iron and/or vitamin preparations. When the final outcome of the pregnancies is studied a very low consumption of these drugs can be recorded among women who will end up having an induced abortion. Women who will have a miscarriage lie between the former group and that of women who will have an infant. Also with respect to psychopharmaca use (8) the mis-

carriage group was intermediate between the induced abortion group and the childbirth group, possibly indicating that the group of spontaneous miscarriages include a number of cases of induced abortions. It could also be shown that the acceptability of pregnancy civil status, social class influenced the use of iron and/or vitamin preparations also within the group of women who later gave birth to normal infants.

The differences recorded for iron and/or vitamin preparations are opposite to those found for analgesics. This could suggest that the recorded incidences are relatively little influenced by mere reporting—women are probably equally apt to remember the usage of these two categories of drugs. Women who will have a dead infant use less iron and/or vitamin drugs than women who will have a living infant especially during gestation.

5-7 There is a similar trend for women who will have infants with major malformations and if the two groups are added together to form one perinatal pathology group the difference compared with the group of women who will have a living normal infant is distinct. Similar findings were reported in a study of 4000 women in Finland (7). Crombie et al (4) also found that women who would have a malformed infant used fewer analgesic preparations than women who would have a normal infant. Nelson & Forfar (17) however found the opposite for the first 56 days of pregnancy. The use of infants with major malformations reported to have used iron more often than women who had normal infants. The retrospective nature of this study may explain at least part of the discrepancy.

A further interesting finding in the present study was that the use of iron and/or vitamin preparations during pregnancy on average gave fewer babies before the 38th week and—in full term babies—higher birth weight compared with the pregnancies when no such preparations were used. It is not clear that this phenomenon is not correlated with the simultaneous use of any other drugs.

It is very unlikely that iron or vitamin preparations especially during the second or third trimester can influence major malformation rates and perhaps it is likely that they can influence infant survival and fetal growth. A possible exception could be folic acid. In a limited but randomized study (18) no effect of iron and/or folic acid supplements during pregnancy on the duration of pregnancy or birth weight was found. The use of iron and/or

preparations is probably mainly an indication of prenatal maternal care and other social conditions which may be of importance (cf 1). Thus smoking habits and nutritional factors could be correlated with the use of these drugs. The observations illustrate that great care has to be taken when conclusions are drawn on drug effects on embryonic or fetal development from mere statistical evidence. A similar situation occurs with anti-cancer drugs and their protective effects against miscarriage as discussed by us in a previous paper (9).

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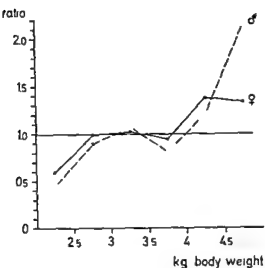


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HISTAMINE METABOLISM DURING THE MENSTRUAL CYCLE

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of Clinical Chemistry (Head Prof S Lindstedt) Sahlgrenska Hospital
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The urinary excretion of histamine and its metabolites methylhistamine (MeHi) and methylimidazoacetic acid (MeImAA) was measured during the menstrual cycle in nine healthy women, one allergic woman and one non-pregnant woman with anovulatory regular menstruation. Simultaneous urinary analyses of luteinizing hormone (LH) and total estrogens were performed. The women showed individual variations in the excretion of histamine, MeHi and MeImAA. This observation is interpreted as an expression of minor individual differences in the catabolism of histamine. At midcycle and in the urinary excretion of histamine metabolites, differences were evident and a statistically significant correlation could be established between MeHi and estrogen in the urine. These results may support previous findings of an increase in the release of estrogens in uterine tissue but may reflect an elevated histamine formation. The allergic woman excreted constantly increased amounts of histamine and its metabolites, especially when her allergic symptoms became aggravated premenstrually. She did not show any change in MeImAA excretion at midcycle. MeHi-excretion varied with the excretion of estrogen in the urine. The subjects with anovulatory menstruation had low values of histamine and metabolites within the normal variations.

metabolites methylhistamine (MeHi) and 1-methyl-4-imidazoleacetic acid (MeImAA) (7) was also demonstrated. No difference has been found between men and women in the urinary excretion of histamine and its metabolites (5-10). However, even under standardized dietary conditions there was a wide range in the amount of MeHi excreted by the females (10). The lowest value was found in a woman using oral contraceptives and the highest in a woman during menstruation. The range in MeHi excretion was considerably narrower in human males. In two women receiving follicle stimulating hormone (FSH) and human chorionic gonadotropin (HCG), a correlation seemed to exist between the ratio of MeHi to histamine in urine and the urinary excretion of estrogens (11). The aim of the present investigation was to study the histamine metabolism more extensively during the menstrual cycle. Special attention was given to the excretion of histamine and metabolites in the pre- and post-ovulatory phases and at the time of the LH peak in order to evaluate any possible relationship to the hormonal variations during the menstrual cycle.

INTRODUCTION

Experimental data indicate that several endocrine factors affect the metabolism of histamine. Sex differences in histamine turnover have been found in the rat (32, 33, 34) and in the mouse (26, 35); the female rat excreting more unmetabolized histamine in urine than the male. During pregnancy a marked elevation in histamine formation is found in these animals and in the hamster (27). The incidence of hormonal influences on histamine metabolism in man is only fragmentary. In human beings, an increased histamine excretion has been reported by several authors (2, 6, 7) and recently a marked increase in the excretion of the histamine

MATERIAL AND METHODS

Altogether thirteen cycles were examined in ten ovulating women. Nine of these women were apparently healthy but one woman had a mild allergic skin disease. For comparison, three anovulatory but regular cycles were examined in three healthy non-pregnant women. Pertinent data on these thirteen women are given in Table I.

During each menstrual cycle urine was collected on as many days as practically possible. The urine was collected in 24-hour portions and immediately placed in a refrigerator at +4°C. Samples for hormone analyses were withdrawn and the urine was then mixed with hydrochloric acid (1 M) to give a pH below 1. On the days of urine

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Table I Pertinent data on the subjects

Subject	Age (y)	Parity	Weight (kg)	Smoking cigarettes/day
A	22	0	54	0
B	22	0	56	>10
C	23	0	82	<10
D	26	1	54	0
E	26	1	58	0
F	46	0	57	0
G	47	1	58	0
H	51	0	74	0
I	53	3	60	0
J	32	1	64	>10
K	26	1	58	0
L	28	0	55	>10
M	49	0	59	0

collection the subjects were given a standardized diet as described by Granerus (10)

Analyses of histamine and metabolites

Histamine (Hi) in the urine was determined by a bio-assay technique according to Wetterqvist & White (36). The values were corrected for recovery and expressed as μg histamine base excreted per 24 hours. The identity of histamine was established in accordance with the method of Reuse (25).

Methylhistamine (MeHi) in the urine was determined according to Fram & Green (5), White (38) and Granerus et al (9). The values were corrected for recovery and expressed as μg base excreted per 24 hours.

1-methyl-4-imidazoleacetic acid (MeImAA) was determined by a method described by Granerus & Magnusson (8) with slight modifications (10). In addition a technique with an internal standard was introduced to increase the sensitivity of the method. A minute amount of ^{14}C labelled MeImAA was added to the urine sample. After the finishing thin layer chromatography the whole material obtained from the cellulose column separation was evaporated to dryness and then dissolved in 1.2 ml aq. dest. One was mixed with 10 ml Instagel (Packard) and the radioactivity measured in a Packard Tri Carb scintillation counter. The quenching effect was estimated in each sample by addition of 50 μl ^{14}C toluol (4.15×10^5 dpm/ml) and recounted. The recovery of ^{14}C toluol was $92\% \pm 3.8\%$ (mean \pm S.D.) and the corrected recovery of added ^{14}C MeImAA was $80\% \pm 6.9\%$ (mean \pm S.D.). By this technique each analysis of MeImAA could be corrected for the losses in the whole analytical procedure and the values were given as mg per 24 hours. ^{14}C MeImAA was obtained by incubation of ^{14}C histamine (The Radiochemical Center, Amersham) and carrier MeImAA with cat liver. The final isolation of ^{14}C MeImAA was performed by ion exchange chromatography (1).

Hormone analyses

Luteinizing hormone (LH) was determined by a radioimmunosorbent technique according to Wide et al (37). The values are expressed in IU per 24 hours in urine and ng per ml in serum.

The total excretion of estrogens in urine was determined according to the method of Brown (3) and Wide (38) and the values are expressed in μg per 24 hours.

Analysis of plasma progesterone was performed and described by Johansson (15) with the modifications suggested by Ellingboe et al (4).

The analyses of estrogens and progesterone were carried out at the Department of Clinical Chemistry, Sahlgrenska Hospital.

Definition of the day of ovulation

In order to verify the day of ovulation the LH peak in urine and the following rise in plasma progesterone were determined. According to Johansson & Wide (15) and Yussman & Taymore (39) and others ovulation is the day after the LH peak. An increase of progesterone in plasma after the LH peak was additional evidence of ovulation (15, 16, 23).

Statistical methods

Conventional statistical methods were used for the calculation of means and standard error of means. A matched correlation analysis based on pairs of observations from each subject—suffer the weakness of correlation intraindividual correlations with very small numbers. With the present method of performance of experiments i.e. longitudinal studies in a few individuals the individual correlation only can be obtained. The product moment correlation coefficient of estrogen and MeHi was calculated for one cycle for each subject. In subject C the first cycle and in subject J the second cycle. By using the correlation coefficients as a sample the positive correlation between estrogen and MeHi was established (two sided tests) by a non-parametric test (4).

RESULTS AND COMMENTS

Ovulatory cycles

Table II gives the mean value S.E. and range of the urinary excretion of histamine and metabolites in each ovulatory cycle studied. In some of the healthy subjects there are relatively high excretion values of histamine and MeHi but it is also evident from Table II that the standard error of the mean is mostly of the same magnitude for each compound. This indicates that different women may have significantly different excretion values of histamine and MeHi. Such an interindividual variation is exemplified by subjects A, B and D who rather constantly had a higher urinary excretion of histamine.

Fig. 1 Urinary excretion of luteinizing hormone (LH), estrogen (Estr), histamine base (Hi), methylhistamine (MeHi) and 1-methyl-4-imidazoleacetic acid (MeImAA) in four ovulating women (A, B, D and G) in two menstrual cycles.

Table II Average daily urinary excretion of histamine base (Hi) and methylhistamine (MeHi) in μg per 24 hrs and 1 methyl-4 imidazoleacetic acid (MeImAA) in mg per 24 hrs during one two or three menstrual cycles in nine healthy ovulating women and one allergic ovulating woman (J)

Compound	No of samples	Mean \pm S.E.	Range
Hi	15	35 \pm 4	21-79
MeHi	15	18 \pm 9	172-747
MeImAA	16	2.63 \pm 0.12	1.60-3.40
Hi	9	60 \pm 4	39-74
MeHi	9	214 \pm 9	184-261
MeImAA	9	3.33 \pm 0.44	2.30-6.50
Hi	17	22 \pm 7	14-78
MeHi	17	271 \pm 2	167-409
Hi	11	23 \pm 3	13-43
MeHi	11	761 \pm 13	211-331
Hi	11	48 \pm 5	24-77
MeHi	11	119 \pm 12	71-206
MeImAA	11	3.71 \pm 0.27	2.0-4.50
Hi	8	25 \pm 3	15-36
MeHi	8	137 \pm 12	99-704
Hi	13	16 \pm 1	17-19
MeHi	13	184 \pm 7	137-744
Hi	17	16 \pm 2	8-31
MeHi	17	134 \pm 8	101-177
MeImAA	17	3.06 \pm 0.18	2.40-4.70
Hi	8	15 \pm 1	17-71
MeHi	8	170 \pm 7	145-702
Hi	11	20 \pm 3	17-39
MeHi	11	117 \pm 15	65-233
Hi	9	31 \pm 3	77-46
MeHi	9	470 \pm 48	268-556
Hi	17	46 \pm 5	20-67
MeHi	17	479 \pm 34	299-660
MeImAA	17	4.96 \pm 0.35	4.20-8.40
Hi	8	50 \pm 9	21-107
MeHi	1	401 \pm 6	279-625
MeImAA	17	3.56 \pm 0.0	2.70-5.10

Anovulatory cycles

Urinary excretion of histamine and metabolites in three women with anovulatory cycles is presented in Table III. The mean values for histamine and MeHi are in the lower range of what is found in ovulatory cycles. The excretion of total estrogen was also somewhat lower. The third case (subject M) was near the menopause as shown by high F values but this did not seem to have any effect on the histamine metabolism as judged from the comparatively low urinary excretion of histamine base (Hi) and MeImAA (Fig. 3). In the other two wom-

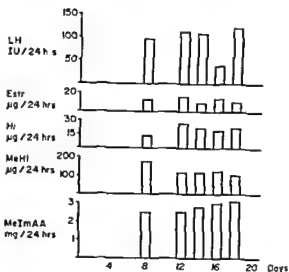


Fig. 3 Urinary excretion of luteinizing hormone (LH), estrogen (Estr), histamine base (Hi), methylhistamine base (MeHi) and 1 methyl-4 imidazoleacetic acid (MeImAA) in a woman (M in Table I) during an anovulatory cycle.

en MeImAA was determined only in a few urinary samples at midcycle which yielded comparatively low values.

DISCUSSION

In man histamine is mainly inactivated by methylation in the imidazole ring and subsequent oxidative deamination, the intermediate metabolite being MeHi and the final metabolite MeImAA. MeImAA

Table III Average daily urinary excretion of histamine base (Hi) and methylhistamine base (MeHi) in μg per 24 hrs in three healthy non pregnant women during one spontaneous anovulatory cycle. In subject M (Table I) the urinary excretion of 1 methyl-4 imidazoleacetic acid (MeImAA) in mg per 24 hrs was also measured.

Subject	Compound	No of samples	Mean \pm S.E.	Range
K	Hi	10	29 \pm 3	17-39
	MeHi	10	118 \pm 11	60-160
L	Hi	11	8 \pm 1	7-17
	MeHi	11	113 \pm 12	50-180
M	Hi	5	21 \pm 2	13-27
	MeHi	5	124 \pm 13	100-180
	MeImAA	5	2.78 \pm 0.17	2.50-3.10

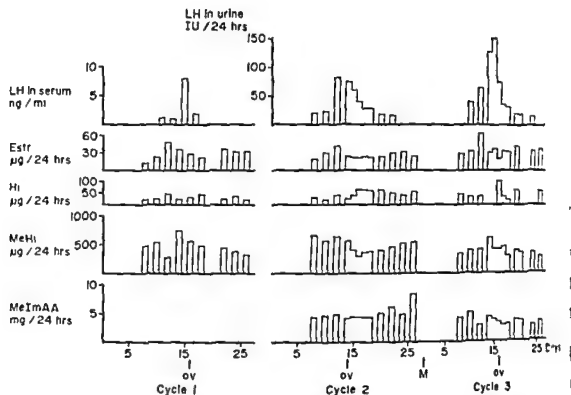


Fig 2 Urinary excretion of luteinizing hormone (LH), estrogen (Estr), histamine base (Hi), methylhistamine base (MeHi) and 1-methyl-4-imidazoleacetic acid

(MeImAA) in an allergic ovulating woman (J) in Table I during three menstrual cycles. During the first cycle LH was measured in serum instead of urine.

lized histamine than any of the other six healthy women. Another example is subject C who excreted small amounts of histamine but had a constantly high excretion of MeHi. This subject was studied during a second menstrual cycle in which she had mean values of histamine and MeHi of the same magnitude as those in the preceding cycle.

The mean value of the quantitatively dominating histamine metabolite MeImAA was about the same in the four healthy women studied (A, B, D, G).

Fig 1 shows the daily urinary excretion of histamine, MeHi, MeImAA, LH and total estrogens in four of the healthy subjects (A, B, D and G) during several days of one menstrual cycle. It will be seen that there are no obvious differences in the excretion of histamine and metabolites in the pre-ovulatory period compared to the post-ovulatory period. However, there was a tendency to an increase in the urinary excretion of MeImAA at midcycle in at least two of the subjects. In subjects D and G the histamine excretion tended to parallel the MeImAA excretion whereas this trend was less evident for the MeHi values.

The complete data from three menstrual cycles in

the allergic woman (subject J) are given in Fig 2. The excretion values of histamine and metabolites were about doubled in this woman as compared to the healthy women (Table II). During the first of the last menstrual cycle studied she had no symptoms but during the second cycle she felt slight tingling of the skin of her hands and arms being exacerbated in the premenstrual phase. The appearance of these symptoms coincided with a marked increase in MeImAA (day 16). No change in MeImAA excretion was found in this woman at midcycle although the MeHi values showed a tendency to increase just before ovulation and a decrease afterwards, i.e. paralleling the variation in urinary estrogens. Determination of LH was not carried out in cases C, E, F and H due to lack of laboratory resources. However, MeHi was determined in all subjects. By using the correlation coefficients as a sample a positive correlation between the urinary excretion of estrogen and LH in subjects A-J was obtained at the 1% level (two-sided tests) by a non-parametric test (Spearman correlation) was found between estrogen and histamine, nor between LH and MeHi or between

laboratory volunteers had a constantly increased histamine turn-over. In clinical practice an increase in histamine metabolism in allergic subjects is often observed before menstruation. This corresponds with the variation in the allergic woman of the present investigation and suggests an increase in histamine metabolism.

It has been shown that in healthy women the excretion of histamine metabolites varies during the menstrual cycle and seems to fluctuate with the plasma levels. As far as we know this is the first report demonstrating a possible connection between histamine metabolism and a normal physiological process in man. Further studies may elucidate whether estrogens trigger a release of histamine or induce an increased histamine formation.

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is quantitatively by far the most important histamine metabolite excreted in the urine and therefore the best measure of the total histamine turnover in the body. However, in order to obtain reliable information about the endogenously liberated histamine it is absolutely necessary to minimize contamination of exogenous histamine by keeping the subjects studied on a standardized diet with a low histamine content (10). All subjects in this study were kept on such a diet each day of urine collection. Under standardized dietary conditions the urinary excretion of MeHMAA is rather constant in healthy human subjects and has been reported to be 2.3 ± 0.3 and 2.4 ± 0.5 mg per 24 hours (mean \pm S.E.) in two different series of non smoking cases (10, 19). The values are somewhat higher among cigarette smokers (10, 31). The mean values of MeHMAA found in the present study agree with the results of these reports except in the allergic woman who had higher values.

The mean values of histamine and MeHi also correspond to earlier reports in healthy human subjects with standardized diet (5, 10) but there were some interesting exceptions. Three of the nine healthy women had a constantly higher output of unmetabolized histamine and another healthy woman with low urinary excretion of histamine had constantly high excretion of MeHi. These findings have been interpreted as signs of minor individual differences in the catabolism of histamine in the body. Catabolic studies with ^{14}C histamine in man have shown a very rigid catabolic pattern of injected histamine in normal as well as in pathological states but this fact does not exclude the occurrence of individual differences as found in the present study (20). The exceptions illustrate the difficulties in evaluating any changes in histamine metabolism from isolated individual observations of histamine and MeHi without standardization of experimental conditions.

It has previously been shown that estrogens rapidly release histamine from uterine tissues in various species including man and that LH releases ovarian histamine in the rat (29). A role for histamine as a mediator of the hormonal vascular effects on these organs has been proposed by Szego (29). The results of the present investigation indicate the occurrence of an increase in urinary excretion of histamine metabolites at midcycle especially MeHMAA. A probably significant positive correlation between the urinary excretion of estrogens and

MeHi was also found verifying the results reported by Green et al (11). No correlation was found between LH and histamine metabolites.

In the rat rapid restoring of the histamine content in the uterus occurs within 14 hours after a subcutaneous injection of estradiol 17β (28, 29). Histidine decarboxylase activity has been demonstrated in the uterus (18). According to Szego (29) variations in endogenous estrogen levels during the estrous cycle of the intact rat was reflected in the uterine histamine content. An inverse relation of uterine histamine concentration and estrus was found. Marshall studied the cycles and found a small decrease in histamine excretion at estrus as well as after administration of stilboestrol but this might be explained by a concurrent depression of urine volume at estrus and during treatment with estrogen (21). By measuring both histamine and MeHi in urine during inhibition of diamine oxidase (Klein & Wetterqvist found no changes in histamine turnover at estrus (to be published).

According to Iversen (14) estrogen treatment increases the number of mast cells in the human ovary and in the myometrium of guinea pigs. Eosinophil cells in blood also decrease in women at midcycle which may reflect an estrogen effect (22, 30).

In mice estrogens primarily increase histamine formation. Injections of estradiol in female mice cause a gradual and long lasting increase in uterine histamine due to a greatly elevated histamine forming capacity of the mouse kidney (17, 27). During pregnancy some species also show a considerable increase in histamine formation (18, 27). In humans however only a small increase in histamine turnover occurs during pregnancy (7, 6, 7). In the present investigation there was evidence of an increase in histamine metabolism in connection with ovulation which might indicate an effect of estrogen on histamine formation or liberation in the human body. It is a well known fact that estrogens exert a selective growth effect upon all the female sex organs. The importance of histamine as a growth factor has been discussed most thoroughly by Kelson et al (18).

It is of interest to note that subjects with regular anovulatory cycles and low estrogen values had normal excretion values of histamine and metabolites. However the anovulatory cases were too few to allow statistical analysis of the data.

The allergic woman who had higher and fluctuating estrogen values in urine than the healthy

EPIDURAL ANALGESIA IN LABOUR

IV Influence on Uterine Activity and Fetal Heart Rate

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Abstract The influence of epidural block with bupivacaine (Marcaine adrenalin®) on fetal heart rate, uterine activity and the frequency and intensity of contractions was studied in twenty-five nulliparae at term. Uterine activity was found to decrease during the first 30 minutes during epidural block. In the time interval 30 to 40 min after epidural block uterine activity increased and attained the same level as during the last 10 min before analgesia. The frequency of uterine contractions did not decrease after the block. The lower intensity of uterine contractions were due only to a lower amplitude of the contractions. The regularizing effect of epidural analgesia on incoordinate uterine action was not observed. The recording of fetal heart rate in the time interval 30 to 40 min after epidural block revealed no pathological findings. Mode of delivery, mean labour duration and Apgar scores after epidural block were comparable with earlier studies of a larger patient population. This study suggests that epidural analgesia does not induce any important changes in fetal heart rate but temporarily decreases uterine activity.

Introduction Continuous lumbar epidural analgesia during labour with bupivacaine as a local analgesic agent has been used since 1969 at the department of obstetrics and gynaecology Karolinska Hospital Stockholm. This study of 25 deliveries under epidural block was undertaken to evaluate the influence of epidural analgesia with a long acting local analgesic upon uterine activity and fetal heart rate.

MATERIAL AND METHODS

Patients Twenty-five healthy nulliparous women in established pregnancy at 37-41 weeks gestation, calculated from the date of the last menstrual period, were selected for this investigation. The age of the patients ranged between 18 and 35 years (mean 23.9 years). All mothers had uncomplicated pregnancies. All babies were singleton and delivered by vertex presentation.

Analgesia The analgesia was started at a cervical dilatation of approximately 4-5 cm. Lumbar epidural block was performed in a conventional way according to a technique described earlier (13, 20). Bupivacaine 0.25% with epinephrine 1:200,000 (Marcaine adrenalin®) was used as the analgesic agent. After an initial test dose of 2 ml, 8 ml were administered through the catheter into the epidural space. This dose was then repeated every second hour until delivery.

Epidural analgesia

Data acquisition At a cervical dilatation of 4-5 cm after membrane rupture a scalp electrode was applied to the head of the fetus. Simultaneously an open-ended polyethylene catheter filled with sterile water was inserted transcervically into the uterine cavity and connected to a calibrated transducer (HP 1280B/C) for evaluating intrauterine pressure. For recording the fetal heart rate (FHR) a Hewlett Packard cardiocograph (HP 8070A) and a Rutgers Spiral Scalp Electrode (16) (HP 15130A) was used.

Fetal blood sampling (FBS) from the scalp was collected according to the method of Salung (18) and the pH was determined on a Radiometer instrument consisting of the blood microsystem BMS 3 and the pH Meter 71.

Definitions Intensity (amplitude) of each contraction is defined as the amount of rise in amniotic pressure above the resting uterine tone. Frequency is expressed as the number of contractions per 10 minutes, calculated from the time interval between peaks of contractions. Uterine activity in Montevideo units (MU) is the product of the intensity of the contractions (in mmHg) multiplied by their frequency and is expressed in mmHg per 10 minutes. Uterine tone is defined as the lowest pressure between successive contractions (11). For evaluating the FHR changes the nomenclature of Hammacher (4) and Hon (6) was used. Changes in FHR patterns in association with fetal blood sampling were excluded.

Table I Clinical data of patients in study

Patient no	Age	Labour duration after block (minutes)	Oxytocin stimulation ^a	Delivery	Apgar score			Weight of infant (g)
					1 min	5 min	10 min	
1	26	256	-	VE	3	8	10	3 460
2	22	280	+	VE	10	10	10	4 400
3	26	235	-	VE	6	8	10	3 140
4	24	139	-	VE	6	10	10	3 550
5	22	54	-	PN	10	10	10	2 810
6	26	254	-	PN	10	10	10	3 60
7	25	79	-	PN	10	10	10	2 970
8	20	64	-	PN	10	10	10	2 570
9	30	800	+	PN	9	10	10	3 340
10	18	118	-	PN	9	10	10	2 740
11	26	567	+	VE	10	10	10	3 220
12	23	297	-	PN	9	10	10	3 420
13	23	250	-	PN	10	10	10	3 600
14	26	254	-	PN	10	10	10	4 370
15	23	141	-	PN	9	10	10	3 380
16	23	275	+	PN	9	10	10	3 410
17	24	145	-	VE	3	5	6	3 35
18	26	281	+	VE	8	10	10	3 020
19	21	232	+	PN	9	10	10	3 440
20	20	119	-	CS	9	10	10	3 650
21	30	397 ^a	-	CS	9	10	10	2 900
22	18	313	+	VE	10	10	10	3 820
23	26	375	+	PN	6	9	10	3 000
24	23	240	+	PN	9	9	10	3 490
25	26	494	+	VE	4	9	10	3 000
x	23.9	267.1						3 330
S D	3.1	170.0						479
n	25	23						25

^a Excluded in mean value of labour duration after block

^a Oxytocin stimulation always was started more than 40 min after epidural block

RESULTS

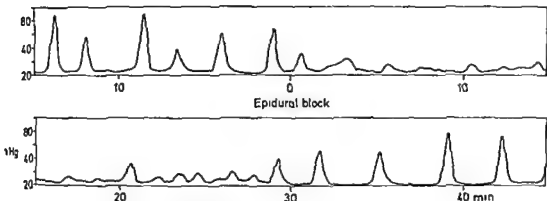
Uterine activity and frequency

The clinical data are presented in Table I. Uterine activity was found to decrease during the first 30 min following epidural block (Table II and Fig. 1). This difference is significant ($0.01 > p > 0.001$). In the time interval 30 to 40 min after epidural block

the uterine activity increased again and attained the same level as during the last 10 min before analgesia. Uterine activity also decreased immediately before analgesia was given. There was even a significant difference in uterine activity 10 to 20 min before the block compared with the last 10 min before analgesia ($0.01 > p > 0.001$).

Table II Intensity (I) in mmHg, frequency (F) in contractions/10 minutes and uterine activity in video units (MU) before and after epidural block

	Before epidural block									After epidural block								
	-30 min			-20 min			-10 min			+10 min			+20 min					
	I	F	MU	I	F	MU	I	F	MU	I	F	MU	I	F	MU	I	F	MU
Mean	34.3	3.7	128.6	32.5	3.7	120.5	27.9	3.6	104.5	19.5	3.6	73.7	20.0	3.6	73.7	20.0	3.6	73.7
S D	11.8	0.9	52.7	11.7	1.0	47.2	9.3	0.9	48.7	8.0	1.1	39.7	9.1	1.5	48.7	9.1	1.5	48.7
S E	2.4	0.2	10.8	2.4	0.2	9.5	1.9	0.2	9.8	1.6	0.3	8.0	1.9	0.3	8.0	1.9	0.3	8.0
n	24			25			25			25			25			25		



1 Influence of epidural block with bupivacaine on uterine activity

If the patients are divided into two groups before epidural analgesia: one group with uterine activity above 100 MU (13 patients) and the other group with uterine activity below 100 MU (12 patients) there will be a more moderate decrease of activity in the first 30 min (mean 149 MU before and 108 MU after 27% decrease) and a more demonstrable decrease in the second group (mean 84 MU before and 54 MU after block 35% decrease).

The intensity of uterine contractions will decrease during the first 30 min after epidural block ($P < 0.05$) in comparison with the 30 min time period before epidural block.

The frequency of uterine contractions does not increase after the block. Lower levels of uterine activity are due only to a lower amplitude of the contractions (Figs 1 and 2).

In two cases incoordinated uterine activity was noted before epidural block. After the onset of epidural analgesia the pattern of uterine contractility became normal (Fig. 3).

In 10 of the 25 cases oxytocin was utilized following the period of observation.

In	F	+40 min		
		MU	I	F
37	96.4	30.5	3.2	104.9
41	33.3	13.9	1.0	49.1
91	10.7	4.8	0.2	10.2
23				

Fetal heart rate (FHR) changes

The recording of FHR in the time interval studied (30 min before and 40 min after epidural block) revealed no pathological findings. In 6 cases early de

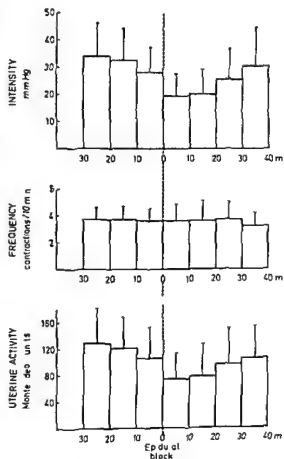


Fig. 2 Influence of epidural block with bupivacaine on uterine activity

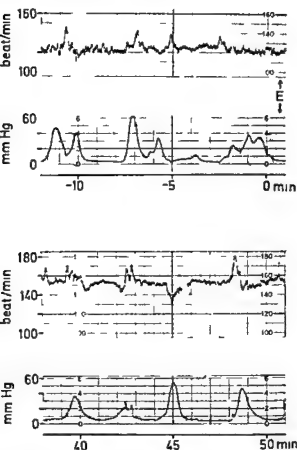


Fig 3 Influence of epidural block with bupivacaine on uncoordinated uterine action. Epidural block at E

celerations were noted before as well as after epidural block. In one case a moderate bradycardia was noted before epidural block and throughout labour. To evaluate these findings FBS was performed 10 and 30 min after epidural block and at a cervical dilatation of 10 cm. The pH was 7.29, 7.28 and 7.24 respectively and the baby received an Apgar score of 10 at birth.

A temporary decrease of the beat to beat variation of the fetal heart rate was observed in 6 cases. In one case an oscillation type O was noted during the 20 min before and the 30 min after the block. 40 min after analgesia the oscillation returned to normal (type 2) and the baby was awarded an Apgar score of 9 at one min.

Fetal blood sampling

In order to evaluate the bupivacaine concentration in fetal blood (1) fetal scalp blood was obtained by the Saling technique in 7 cases 20 min after analgesia and in 8 cases 10 and 30 min after epidural block.

The pH was normal in all cases and ranged between pH 7.27 and 7.42.

Delivery and condition of the newborn

Mode of delivery: mean labour duration after epidural block and Apgar scores of these 23 cases were comparable with earlier studies of a larger population (13). In one case vacuum extraction was performed because of a severe umbilical cord complication. The other vacuum extractions were performed due to secondary uterine inertia. The indication for caesarean section was in case 70 a severe umbilical cord complication and in case 71 a narrow pelvis.

DISCUSSION

The results of this study indicate that there is a significant decrease in uterine activity during the initial 30 min after epidural block. This is manifested primarily by a reduction of the intensity of the contractions without any change in frequency. This phenomenon has been described earlier (8, 17, 21) when lidocaine, mepivacaine or procaine were administered. This investigation has clearly shown that this is also the case when bupivacaine is used. In addition, this study indicates that epidural block will depress the amplitude of the contractions when given during the early stage of labour. In well-established labour the depressive effect on uterine activity is more moderate. In both groups oxytocin stimulation will however be correct. Shorter acting drugs such as lidocaine must be given more often and consequently they should influence the duration of labour more than longer acting drugs such as bupivacaine. Since a temporary decrease of uterine activity is seen after each injection, if lidocaine is used a continuous drip method might be more convenient (22, 23).

The regularizing effect of epidural analgesia on uncoordinated uterine action is well known (9, 10, 24) and has been observed also during this study. Following epidural block and a period of depressed uterine activity, the contractions return to normal even without oxytocin stimulation. In practice it can be difficult to stimulate a patient with uncoordinated uterine activity without good pain relief as this indication for epidural block is therefore of great importance.

The decreasing uterine activity before epidural block is more difficult to evaluate. Prior to the block

patient is in the supine position but thereafter the lateral position just before epidural analgesia. This change in position might cause reuterine activity. With the technique used in this investigation the evaluation of uterine tonus is totally accurate but a pathological rise in tonus has not been recorded.

The changes of FHR such as early decelerations were found in this series. This pattern can be considered normal (6-7). No pathological periodic changes or fetal bradycardia as reported to occur after paracervical block (15-19) have been observed after epidural block in this study. In 6 cases a decreased oscillation of FHR was noted after epidural analgesia. Statistical evaluation of this could not be performed due to the relatively small number of cases. A similar observation has been reported earlier (5-14) and in another study (2) it was shown that decreased FHR oscillation was not associated with high fetal bupivacaine levels. This pattern is a rather nonspecific one which for example can be elicited by morphine, pethidine and diazepam (3). Some authors (10-14) have reported pathological FHR changes after epidural block but these findings could not be confirmed in the present study. A difference in dosage or fetal bradycardia due to maternal hypotension could explain the different findings. This study suggests that epidural analgesia does not induce any important changes in fetal heart rate and rather effects a temporary decrease in uterine activity. In our opinion these findings do not represent any drawback when lumbar epidural block in labour is needed for effective pain relief.

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SURVEY RADIOGRAPHY OF ABDOMEN FOLLOWING CAESAREAN SECTION WITH PARTICULAR REFERENCE TO CAECAL DIAMETER AND THE PRESENCE OF FREE SUBDIAPHRAGMATIC GAS

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The literature concerning perforation and immu-
rforation of the caecum is reviewed. The greatest
nce is attached to 5 cases of perforation of the
associated with paralytic ileus following Caesarean
1. The caecal diameter was determined by radio-
1 in 100 patients on the 3rd and 5th day after
rean section. At the same time the volume of sub-
apmatic free gas was assessed. An average caecal
ter of 6.4 cm was found which is not different from
idings in the normal population. Increasing caecal
ter during the puerperium gives rise to suspicion of
ecum. In 90% of the patients free subdiaphragmatic
appeared by the 5th day after Caesarean section.

ration of the caecum with overdistension
ed by obstruction of the distal colon has been
bed many times in the literature (1 10 14 16).
aneous perforation of the caecum in connec-
with paralytic ileus where no mechanical ob-
tion could be found during surgery is ex-
tremely rare (7 12 22).

requently perforation occurs on the anterior
ne of the caecum 5 cm distal to the ileo-caecal
junction. Based on clinical and experimental studies
theories have been advanced as to the reason
for this site and there are several
etiological factors (5 10 16 19 20 21).

clinical symptoms of perforation and immu-
perforation of the caecum are often non-
specific and few (1 5 14 16). In order to
make a radiological measurement of the largest
ter of the caecum in cases of obstruction of the
colon Lowman & Davis Davis & Lowman (1957)
studied 17 cases with imminent perforation and 7
caecal perforation. In these 19 patients they

found caeca varying in diameter between 9.0 and
16.3 cm. In a series comprising 100 subjects without
previous intestinal disease colon examinations with
barium and air insufflation were made. The average
greatest caecal diameter was 6.5 cm and in 97 the
diameter was less than 9 cm. Lowman & Davis
concluded that a caecal diameter of 9 cm or more
indicated imminent intestinal perforation and in
these patients surgical intervention would always be
necessary. In cases described in the literature the
surgical treatment in imminent perforation and
caecal perforation was mainly caecostomy (1 10 12
16). The mortality in connection with caecal perfo-
ration is high varying between 44% and 72% (1 10).
Lowman & Davis found a mortality of only 8% if
surgery was carried out before perforation occurred
(12 cases).

Several reports are available on colonic obstruc-
tion with distension of the caecum and caecal perfo-
ration following normal delivery and Caesarean sec-
tion (2 3 8 13 17). Only 5 cases of caecal perfo-
ration associated with paralytic ileus following
Caesarean section have been reported (6 9 11 15).
In these 5 patients the symptoms were few and
non-characteristic as in other acute abdominal dis-
orders occurring during the puerperium. Common
features in all were raised temperature raised pulse
rate varying abdominal distension and pains mainly
in the right iliac fossa. In none were there clinical
signs of mechanical obstruction. Only one patient
presented with signs of irritation. Only in the case
described by Kålund Jensen was a caecal diameter of
10 cm revealed on repeated examination of the
radiographs. Based on the case reports it is difficult

Table I Indications for Caesarean section in 100 patients

Cephalopelvic disproportion	21
Incoordinate labour	13
Malpresentation	11
Prolapse of cord	10
Asphyxia	9
Previous caesarean section	7
Placenta previa	6
Abruptio placentae	6
Elderly primigravida	6
Toxaemia	3
Other causes	8
Total	100

to ascertain the interval between the caecal perforation and surgery but in 3 patients (9-11) free gas under the diaphragm was present on the 5th day after Caesarean section and surgery was not performed until the 6th or 7th day. In these the free gas was taken to be caused by the preceding Caesarean section. However, it has previously been proved that air introduced into the abdomen at laparotomy is usually absorbed within 5 to 7 days (11-18).

These 5 cases of caecal perforation and paralytic ileus after Caesarean section in which the symptomatology was limited and the surgical findings surprising, stimulated the present investigation to determine the greatest caecal diameter following normal Caesarean section and to determine how quickly residual air in the abdomen disappears after Caesarean section.

MATERIAL AND METHODS

The study comprised 107 patients who had undergone Caesarean section over a one year period in the maternity ward at St. Joseph's Hospital. The indications for Caesarean section are shown in Table I. Fifty of the 107 patients were primigravidae. Their ages varied from 17 to 41 years with an average age of 27 years. The group studied comprises all patients with a normal course of

Table II Caecal diameter measured on the 3rd and 5th day after Caesarean section

Day	Number	Mean (cm)	Range (cm)	S.D.
3	99	6.7	4.9-9.5	0.99
5	97	6.0	4.3-8.5	0.08

S.D. Standard deviation

Table III The amount of free gas in the abdomen measured on the 3rd and 5th day after Caesarean section

Day	Number	Mean (cm)	Range (cm)	S.D.
3	100	0.16	0-1.0	0.37
5	100	0.07	0-0.4	0.28

Caesarean section and puerperium. Two patients were excluded: one of them underwent appendectomy on the 5th day after Caesarean section and for the other radiographs were not satisfactory. 76 were elective section and 74 patients were in labour.

As anaesthesia for Caesarean section a combination of succinylcholine, thiopental sodium with nitrous oxide and oxygen was used.

On the 3rd and 5th day after Caesarean section survey radiographs of the abdomen were taken in the supine position. Each radiograph showed the diaphragm, liver and the caecal region. The assessment of the greatest caecal diameter without using contrast agent presented any difficulties. In 3 patients it was not possible on the radiograph to locate the caecum because of too little gas and no fluid level.

The amount of free gas in the abdomen was measured as the greatest border of gas under the diaphragm.

RESULTS

Table II shows the average greatest diameter of the caecum on the 3rd and 5th days after Caesarean section. There is no statistically significant difference in the caecal diameter measured on the 3rd and the 5th days at 95% level (Student's *t* test).

On the 3rd day 4 patients had a caecal diameter ranging between 9 and 9.5 cm. None of these presented any clinical signs of mechanical obstruction but one was treated with suction and paracentesis of fluids because of vomiting. Three of the 100 patients vomited during the puerperium and were therefore treated for short periods with suction and paracentesis of fluids. On the 5th day all patients were comfortable well.

On the 3rd day following Caesarean section a survey radiograph in 26 patients showed no free fluid levels but only 4 of these presented a small picture on the 5th day. In none of the patients had the caecal diameter increased by the 5th day. Furthermore there was no decrease.

In the group of patients where elective Caesarean section was used nobody had signs of paralytic ileus.

the puerperium and all had a caecal diameter less than 9 cm

54 patients no free gas in the abdomen could be demonstrated radiologically on the 3rd day after Caesarean section and on the 5th day 90 patients had no free gas in the abdomen. In all patients the amount of subdiaphragmatic gas was less on the 5th day as compared with the 3rd day.

DISCUSSION

In the present study an average caecal diameter of 6.5 cm was found. No significant difference in average caecal diameter was found between the 3rd and 5th day after Caesarean section. In their control study Lowman & Davis found an average caecal diameter of 6.5 cm.

On the 3rd day after Caesarean section only 4 patients had a caecal diameter of 9 cm or more and none of them presented specific or particularly pronounced clinical symptoms. One was treated with enema and parenteral fluids. All the patients had increased caecal diameters on the 5th day after Caesarean section. Lowman & Davis stated that a caecal diameter of 9 cm or more indicated imminent perforation of the caecum and concluded that surgical intervention would be necessary in such cases. One of the 4 patients with a caecal diameter of 9 cm or more was treated with enema. Kälund Jensen advised against enema in cases of paralytic ileus because of caecal distension because a pressure of 100 cm H₂O is commonly used to introduce an enema and this pressure might be transmitted to the caecum causing perforation. Millar & Øvlisen (11) found this assumption to be of importance because on various occasions caecal perforation had been observed both clinically and experimentally at intra-abdominal pressures of from 20 to 50 cm H₂O (19–21).

Our study showed that the amount of free gas left in the abdomen after Caesarean section is very small. In only 50% was it possible to find a slight amount of free gas in the abdomen on the 3rd day after Caesarean section. In 90% no gas was evident on the 5th day.

CONCLUSION

Survey radiography of the abdomen without using contrast media in the colon is well suited for ascertaining the caecal diameter after Caesarean section.

During the puerperium after Caesarean section the average caecal diameter was 6.4 cm. This does not differ from previous findings in the general population.

In patients with distension of the abdomen and increasing caecal diameter during the puerperium after Caesarean section the danger of caecal perforation should always be borne in mind. At the same time it must be remembered that the amount of free gas in the abdomen following Caesarean section is always very small and will have disappeared in 90% of patients by the 5th day after operation.

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THE USE OF ISOXSUPRINE IN ESSENTIAL DYSMENORRHEA

A controlled clinical study

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Abstract The uterus specific beta adrenoceptor stimulant isoxsuprine was used for the treatment of primary dysmenorrhea in a double blind controlled clinical study. No significant beneficial effect of the drug was identified.

beta adrenoceptor stimulating agents are frequently administered in an attempt to inhibit premature labor. Theoretically it might be expected that these agents could be of some use in the treatment of dysmenorrhea. Dysmenorrhea is listed as an indication for the use of isoxsuprine (Cardilan, Ferro-T) in the Norwegian drug index (Felleskatalogen). However, apart from one uncontrolled study (2) no clinical study has been undertaken to see whether isoxsuprine really is effective in the treatment of dysmenorrhea. A controlled double blind clinical trial of isoxsuprine against placebo in the management of dysmenorrhea is reported here.

palpitations, dizziness, headache, tendency to faint, nausea were especially mentioned to them. 3) When menstruation started related to when they started taking the tablets. 4) Whether the menstrual loss was heavier or lighter than usual. 5) Whether they wanted to go on using the tablets.

RESULTS

Twenty six of the participants completed the trial. One of those who did not complete did so because of side effects of the first course of tablets (which was placebo). For the other three the reasons were not related to the tablets.

Of the 26 who completed, 14 did not have any relief of dysmenorrhea either with isoxsuprine or with placebo. One person had relief of pain both with isoxsuprine and with placebo. Seven had relief of pain with isoxsuprine and not with placebo, while 4 had relief of pain with placebo and not with isoxsuprine. This difference is not statistically significant. Of the persons reporting relief of pain, only one reported complete relief, while the others reported some relief.

Six persons reported side effects: 5 with isoxsuprine and one with placebo. The side effects of isoxsuprine were dizziness (2), quivering and dizziness (1), nausea (1) and headache (1).

Whether isoxsuprine had been taken for two or more days before the onset of menstruation or had been started on the same day as the bleeding was not related to its effect.

There was no tendency for isoxsuprine to prolong the menstrual cycles. No significant change in the amount of menstrual loss was noted.

Three of the 8 persons who apparently benefited from isoxsuprine indicated that they wanted to go on taking the tablets, and all of the 5 persons who had benefited from placebo wanted to go on taking those.

MATERIALS AND METHODS

Subjects Medical and pharmacy students were asked to volunteer for the project. 30 students were enrolled: 19 were nulliparous, 1 primiparous. One had an IUD, the others used other types of mechanical contraception. 18 had severe dysmenorrhea. 12 of these had to stay in bed for one day each period. The other 12 had moderate dysmenorrhea, which means that they could attend lectures etc.) with the use of analgesics. The participants were instructed to take tablets for two days before the beginning of menstruation and for as many days as they usually had pain. The tablets were isoxsuprine 70 mg x 3 or placebo tablets identical in appearance. Two consecutive menstrual cycles were used: one with isoxsuprine and one with placebo for each participant. The type of tablets taken during the first cycle was randomized. The participants were told that some of them might get placebo tablets, but they did not know that each of them would get one cycle with the active drug and one with placebo. The participants were questioned about the following: 1) The effects of the drug. 2) Side effects (the options

DISCUSSION

This investigation shows that isoxsuprine has no significant effect in the relief of essential dysmenorrhea. While this study was in progress the same results were reported for hydroxyphenyl orciprenaline (Berotec) another beta adrenoceptor stimulating agent (1). It must be concluded that beta adrenoceptor stimulation is ineffective in relieving menstrual pain.

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CERVICAL BACTERIAL FLORA IN WOMEN FITTED WITH A COPPER RELEASING INTRA UTERINE CONTRACEPTIVE DEVICE (IUD)

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Abstract A bacterial culture was taken from the cervix in sexually active women before and 3 and 6 months after insertion of either a copper releasing or an inert intra-uterine contraceptive device (IUD). Sixty had a Copper T (TCu 200) and 25 a Lippes loop D. Although more than a quarter of the patients the bacterial flora changed slightly in diversity and abundance after IUD insertion, there was no difference in effect between the TCu 200 and Lippes loop D.

Intra-uterine infection, septic abortion and septicemia have been attributed to the use of intra-uterine devices (IUDs). Despite the wide spread use of this method of contraception there have been reports of serious pelvic infections with a conventional inert IUD *in situ* (6). Recently however the Dalkon Shield has been reported to cause sepsis and even fatal infections during the second trimester of accidental pregnancy (1, 6, 9). The filament string unique to the Dalkon Shield, by capillary action, provides a mechanism for pathogenic bacteria from the vagina entering the uterine cavity (13). Mishell et al. (7) showed by transfundal sampling that there were bacteria in the uterine cavity after insertion of an IUD (Lippes loop). However, the incidence of positive cultures diminished rapidly and all endometrial cavities studied were free of bacteria within one month after insertion. Other studies have also shown that the presence of inert IUDs does not increase significantly the incidence of positive cultures in the endometrial cavity (17, 18). Facina et al. (3) demonstrated the gonococcicidal action of copper *in vitro*, but this observation has

not been confirmed *in vivo*. In fact Spellacy et al. (11) found that copper releasing IUDs do not prevent gonococcal infection. The aim of this study was to investigate whether copper releasing IUDs have an adverse effect on the cervical bacterial flora under *in vivo* conditions.

MATERIALS AND METHODS

Eighty-five healthy parous and sexually active women who had requested IUD insertion were chosen for study. The Copper T (TCu 200) group consisted of 60 women and the Lippes loop (type D) group, which served as a control of 25 women. The age structures of the two groups are shown in Table I.

All IUDs were inserted postmenstrually. Before insertion a cervical swab was taken for bacterial culture and a Papanicolaou smear and vaginal swab for yeast and *Trichomonas vaginalis* screening. The same examinations were repeated 3 and 6 months after IUD insertion.

The cervical swab was sent to the laboratory in a transport medium (modified Stuart medium ref. 8). The swab was cultured on normal blood agar plates in 5% defibrinated sheep's blood, on a lactose agar plate, on a choco-

Table I Age distribution in TCu 200 and Lippes loop D groups

Age	TCu 200 (%)	Lippes loop D (%)
20-25	2.6	6.6
26-30	27.6	23.3
31-35	47.1	26.6
36-40	23.6	10.0
41-45	3.9	26.6
46-	1.3	3.3

Table II *Bacteria possibly present in normal vaginal flora*

Döderlein bacillus groups
Apathogenic spirochaetes
Diphtheroids
<i>Staphylococcus albus</i>
Non haemolytic streptococcus
<i>Streptococcus viridans</i>
<i>Escherichia coli</i>
<i>Streptococcus faecalis</i>
<i>Staphylococcus aureus</i> sometimes
<i>Mycoplasma hominis</i> types I and II

late agar plate and on a medium for gonococcus (gc medium) (10-14). After these cultures had been set up the stick was used to inoculate a liver broth tube. The plates were incubated at 36°C, the chocolate agar and gc medium plates in the presence of CO₂ (from 5% to 10%) and examined after 24 and 48 hours. The extent of bacterial growth was estimated and recorded on scale from + to ++++. The bacteria were identified by standard methods (4) and the drug sensitivities of the different types were determined by Ericsson's disc technique (?). A culture was considered negative when even in the liver broth medium no growth was observed after 72 hours incubation.

The bacteria seen in vaginal cultures from apparently healthy women are listed in Table II.

RESULTS

All the patients were free from *Neisseria gonorrhoeae* before insertion of the IUD and no gonorrhoeal infection was found in either group at check-ups after 3 or 6 months. During the study one positive *Trichomonas vaginalis* culture was found in each group at the 3 month check-up. The patients with trichomonal infection were treated with metronidazole (Triazolol® Medipolar Oy Oulu, Finland) 200 mg three times daily for 7 days by mouth (the male partner was treated likewise) and locally with povidone iodine (Betadine® Leiras Oy, Turku, Finland) for 10 days. Yeast infection was more common in the Copper T group both before

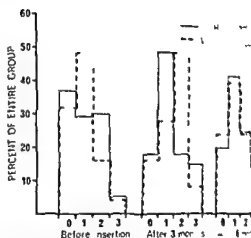


Fig. 1 Proportions of women harbouring more bacteria in the cervix before insertion of the IUD and 3 and 6 months later.

insertion (6 cases) and during the course of study (Table III). *Candida* infection was treated locally either with nystatin vaginal tablets 100,000 IU (Nystacid® Läike Oy, Turku, Finland) or with natamycin vaginal tablets 25 mg (Pimarol® Mycofarm, Delft, Holland) at night for 7 weeks. In the routine Papanicolaou smears there were no signs of malignancy.

The events associated with the use of the IUDs are recorded in Table IV. There were no accidental pregnancies in the Lippos loop D group (one ectopic for which salpingectomy was performed and one spontaneous abortion with the Lippos loop D *in situ*) but none in the Copper T group. No patients showed any signs of pelvic inflammatory disease (PID).

The results obtained from the cervical cultures grouped according to the number of different types of bacteria before insertion and 3 and 6 months afterwards are shown in Fig. 1. The number of bacteria increased slightly in both groups.

As judged from the growth in the cultures 15 patients (26.6%) in the Copper T group and 7 (11.8%)

Table III *Trichomonas vaginalis* and *Candida albicans* cultures from vaginal swabs before and 3 and 6 months after insertion of TCu 200 and Lippos loop D

	Number of patients	<i>Trichomonas vaginalis</i>			<i>Candida albicans</i>		
		0	3	6	0	3	6
Lippos loop D	25	—	1	—	—	5	1
TCu 200	60	—	1	—	6	14	10

Table IV Termination associated with six months use of TCu 200 and Lippes loop D by the respective groups

	TCu 200	Lippes loop D
Number of patients	(60)	(25)
Irregularity	—	2
Spotting	2	—
Pain and/or bleeding	1	2
Personal reasons	1	—
Total	4	4

the Lippes loop group had a more abundant bacterial flora after 6 months of an IUD use than before insertion. The resistance of the bacteria to antibiotics and chemotherapeutic agents did not differ during the course of the study.

DISCUSSION

It has earlier suggested that copper and its salts have bactericidal activity (12). Of the microorganisms tested *in vitro* the only one that was sensitive to cupric salts was *Neisseria gonorrhoeae* (13). As we found no differences between the Copper T and Lippes loop groups 3 and 6 months after insertion our study confirms that copper *in vivo* has no adverse effect on the cervical flora.

Although the cervical bacteria are slightly more abundant after insertion of an IUD no cases of PID were found in this study. By direct transfundal sampling Mishell et al (7) showed that the endometrial cavity is highly resistant to single non-pathogenic bacterial contamination: some 80% of women they studied were already bacteria negative within 48 hours of IUD insertion. In our two large-scale studies (15, 16) for the evaluation of Copper T 200 and Copper T 300, where a total of 109 Copper T insertions were made and more than 44 000 women months of use monitored, the infection rate during the first year of use estimated by the life table method was only 1%. In the whole series only one severe case of PID was encountered.

Our study confirming the results of previous microbiological evaluation (5) demonstrated that all bacterial vaginal bacteria were slightly more abundant in IUD users than among other women; there was no difference between the inert and copper releasing IUD groups.

The study also shows that during IUD use the resistance of these bacteria to antibiotics and chemotherapeutic agents does not alter.

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INDUCTION OF THE PREGNANCY ZONE PROTEIN BY CONJUGATED OESTROGENS

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Treatment with conjugated oestrogens was significantly the serum concentration of pregnancy zone protein (PZ) in a group of postmenopausal women. The basic level of PZ before treatment was comparatively high in this group of women and related to age and previous pregnancies. Naturally occurring conjugated oestrogens have a similar effect on the induction of PZ as synthetic oestrogens.

The induction of the pregnancy zone protein (PZ) is believed to be oestrogen-dependent (5, 6, 17). Concentrations about 100 mg/100 ml of this high molecular weight serum α_2 -glycoprotein (4, 13, 14, 18) are found during late pregnancy and after delivery there is a rapid decrease to nonpregnant levels (<4 mg/100 ml) (15).

Exogenous administration of synthetic oestrogens is also known to increase the serum concentration of PZ in nonpregnant women and men (1, 2, 6, 11). The induction of PZ seems to be affected by the type and dosage of treatment (2, 6). In a previous study of women treated with combined oral contraceptives 1 mg norethisterone + 0.1 mg mestranol (Norgestrel) was found to be a more potent inducer than 0.5 mg norgestrel + 0.05 mg ethinyl oestradiol (Follinyl®) while 0.3 mg norethisterone (Minipil®) gave no measurable increase of PZ (6). When given to men with prostatic cancer a combination of polyoestradiol phosphate (Estradurin®) and diethylstilboestrol (Stilbol®) or diethylstilboestrol only was found to induce PZ while polyoestradiol phosphate only gave no measurable increase (7).

Previous to the effect on the synthesis of PZ of naturally administered oestrogen has been investigated using synthetic oestrogens. Conjugated oestrogens are used in treat-

ment of oestrogen deficiency in postmenopausal women and the side effects on blood coagulation (9), liver function (3) and lipid metabolism (12) are believed to be less pronounced than with synthetic oestrogens. Little is known about the influence on the synthesis of PZ.

The recent development of a highly sensitive radio-immunoassay for PZ (7) has made it possible to study the low levels of this protein in untreated nonpregnant women. Most previous studies on this protein have been made on young women taking oral contraceptives or on women during pregnancy. In the present investigation the concentration of PZ was measured by radio-immunoassay in postmenopausal women before and during treatment with conjugated oestrogens.

MATERIAL AND METHODS

Venous blood samples were drawn from women at ending the Department of Obstetrics and Gynaecology, University of Umeå, for postmenopausal symptoms: profuse sweatings and hot flushes being the commonest. 34 women were investigated before treatment. All women were apparently healthy, taking no other drugs. 11 of these women were investigated also after two months of treatment with 1.25 mg Promant® six days per week. Promant® contains naturally occurring conjugated oestrogens: mainly oestrone sulphate and equilin sulphate prepared from pregnant mare urine.

After coagulation all sera were decanted and stored at -70°C until use. Samples were coded and tested blindly in duplicate. The concentration of PZ was determined by a radio-immunoassay (7) based on determination of 125 I-labelled PZ using monospecific rabbit antiserum and the purified protein as a standard (13, 14). Separation of free and antibody bound PZ was performed with the double antibody solid phase method (DASP) (10) and the range for measurements was 50-2000 ng PZ/ml (7).

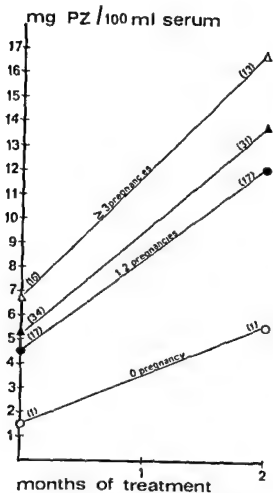


Fig 1 Mean concentration of the pregnancy zone protein in sera of women before and after two months treatment with conjugated oestrogens (Promant®). The total material (▲—▲) is divided according to previous pregnancies. Number of women is indicated within parentheses.

RESULTS

Some characteristics of the investigated women are presented in Table I.

All women were found to have measurable basic levels of PZ, and an increase in PZ concentration was found in all individual women after two months of treatment. The mean serum concentration of PZ in 34 women before treatment was 5.3 ± 0.6 mg/100 ml (mean \pm S.E.M.). After treatment with conjugated oestrogens (Promant®) for two months the mean serum concentration for 31 women was 13.9 ± 1.7 mg/100 ml (mean \pm S.E.M.) (Fig 1). The increase was significant ($P < 0.01$).

The women were grouped according to previous pregnancies as shown in Fig 1. The mean value of the concentration of PZ before treatment for 16

women with 1-2 previous pregnancies was 4.5 ± 0.3 mg/100 ml. The corresponding figure for women with 3 or more pregnancies was 6.7 ± 1.1 mg/100 ml. For both these two subgroups the increase of PZ after two months of treatment was statistically significant ($P < 0.01$) and reached levels of 12.2 ± 1.5 and 16.9 ± 3.3 mg/100 ml respectively. The woman with no previous pregnancy increased her PZ concentration from 1.5 to 5.6 mg/100 ml.

DISCUSSION

During treatment with conjugated oestrogens (Promant® 1.25 mg daily 6 days per week) all women increased their concentration of PZ from two up to five times. This study thus gives further evidence for an oestrogen dependent PZ-synthesis.

All women had measurable amounts of PZ before treatment and the basic levels were high when compared to levels earlier observed in young women (15-25 years of age) with basic PZ concentrations below 1 mg/100 ml (8).

As shown in Fig 1 the number of previous pregnancies might be of importance for the basic level of PZ and may furthermore affect the concentration of PZ after hormonal treatment. The material is, however, too small to allow definite conclusions. Present studies of the effect of age and previous pregnancies on the PZ concentration seem to confirm these results (8).

This study indicates no straight dose-response relation between the oestrogen level and the basic PZ concentration. The group of elderly women with symptoms of oestrogen deficiency shows high amounts of PZ than younger women. The observed increase in PZ concentration is lower than during pregnancy when the mean level in the last trimester is around 100 mg/100 ml (15). The duration of treatment was, however, only two months. Data from treatment with combined oral contraceptives level between 15 and 50 mg/100 ml can be seen (16).

Table I

Number of patients before treatment	34
Number of patients after two months of treatment	31
Mean age in year \pm S.D.	41 \pm 4.9
Interval since last menstruation	4 months-8 years

treated with conjugated oestrogens for post symptoms induce PZ in concentrations which are equal to those observed for young women with combined oral contraceptives. Product which only contains naturally occurring oestrogens acts in this respect similar to synthetic oestrogens.

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IN VIVO DETERMINATION OF THE STRESS-STRAIN RELATION OF THE HUMAN MYOMETRIUM

Ingeemar Joelsson Lennart Gidlund Bo Anzen and
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Smooth muscle is capable of responding by contraction to mechanical distension. Neither the structural basis for this phenomenon nor the theories of smooth muscle behavior are completely clear. A method is now described by which the contractile response to mechanical distension of the human uterine muscle in vivo is examined. The distension is enforced sinusoidally within a frequency range 0.005-2 Hz by filling and emptying a thin walled rubber balloon introduced into the uterine cavity. A stress-strain index of the myometrium is defined using a law after the approximate evaluation of a contractile modulus and the tangential stress and strain in the inner layer of the uterine wall. The regression line of mean stress upon frequency is calculated and intercept and regression coefficient of the equation for basic conditions compared with those obtained during administration of pharmacologically active agents. It is suggested that the differences in intercepts and regression coefficients be used as a measure of drug effect.

Experiments on intestinal smooth muscle do support the contention that the sliding filament theory which quantifies a length active tension relationship can be extended to smooth muscle (7). But there is a wide variety of muscular behavior which does not conform to any of the well known hypotheses of muscular function. Examples are phenomena such as stress relaxation equivalent to adaptation following step-wise strain and stress response to sinusoidal strain. A model is therefore needed which accounts for mechanical properties on the basis of physical as well as chemical changes. Pending this a technique has been designed which might serve as a tool for the classification of parameters to be looked for in the determination of drug effects on the uterine muscle.

TECHNIQUE

The effect upon the uterine muscular wall of pharmacologically active agents has most often been expressed in terms of modification of intracavitary pressure amplitude and frequency of contraction. Intrauterine pressure is however a complex function of the tension within the muscular wall, its curvature and thickness. In addition spontaneous changes in myometrial activity complicate the evaluation of drug effects on the basis of pressure measurements calling for greater insight into the modification of muscular responses to well defined specific stimuli. Strips of smooth muscle tested in vitro as well as whole muscle in vivo are capable of responding to variation to mechanical distension. Neither the structural basis for this phenomenon nor the theories of smooth muscle behavior are completely

The aim has been to record and analyze the development of tension in the uterine myometrium as a contractile response to distension. The filling and emptying of a thin walled rubber balloon placed in the cavity of the organ was chosen to induce the mechanical stimulation. The balloon serves as a receptacle for predetermined quantities of fluid pumped into and withdrawn from it in sinusoidal sequences. Proportional distension of the wall of different sized uteri is achieved by using balloons and matching pump systems of varying dimensions. Each of these units has been manufactured to cater for the influences of dynamic processes on the measurement of pressure.

A bellows pump is connected to a transmission tube ending in a stem with several perforations (Fig

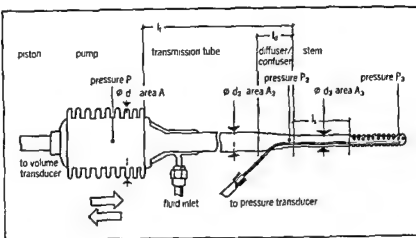


Fig 1 Mechanical device for uterine distension of the uterus. The thin walled rubber balloon mounted without leakage at the perforated part of the stem

1) Around the stem to be placed inside the uterus a thin walled rubber balloon is attached and checked for the absence of leaks. Inside the intra uterine stem and partly within the transmission tube a cannula with a small diameter is placed. This cannula tube is used for pressure transmission and ends in one of the perforations to avoid disturbances from the streaming fluid. All the other openings in the stem serve as connections between the interior of the stem and the interior of the balloon.

The bottom of the bellows is connected to a piston governed by an eccentric mounted on the end axis of a shunt wired electric motor of DC type with constant voltage over the field but not over the armature. Variable speed transmission within the range 0.005–10 rotations per second is achieved with a thyristorized current control and a two-step mechanical gear. The feed back of a tachometer generator ensures a constant speed at each setting. Mechanical connection between the bottom of the bellows and a linear precision potentiometer effects the monitoring of volume in the balloon as a function of time on one channel of a dual beam storage oscilloscope. The instantaneous pressure in the balloon which does not take any tension is considered equivalent to the overall pressure in the uterine cavity and recorded on the other channel of the oscilloscope by means of a pressure transducer.

HYDRODYNAMIC CONSIDERATIONS

The following requirements have guided the dimensioning of each unit

1 The entire system shall be inelastic and must not contain gas or vapor at any time

2 The intracavitary pressure shall be determined correctly

To achieve this the instantaneous change of volume in the balloon must for example be synchronous with the change of volume in the pump. Furthermore pressure losses which from either friction or inertia during movement of fluid in the system shall be minimized.

EQUATION SYSTEMS

V_0 is defined as the bellows displacement from middle position to one extreme. The volume of the intrauterine balloon (V) is a function of time according to

$$V = V_0 \sin \omega t$$

ω being the angular frequency in radians/s, the volume flow (Q) is determined by the equation

$$Q = \frac{dV}{dt} = V_0 \omega \cos \omega t$$

and volume flow acceleration (\dot{Q})

$$\dot{Q} = \frac{d^2V}{dt^2} = -V_0 \omega^2 \sin \omega t$$

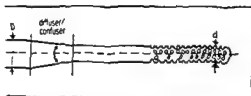
The flow velocity (v) in any part of the area A_1 is

$$v = \frac{1}{A_1} V_0 \omega \cos \omega t$$

and flow acceleration (a)

$$a = -\frac{1}{A_1} V_0 \omega^2 \sin \omega t$$

Prerequisites for the validity of equations (5) are that the system is rigid, the fluid in-



2 The construction of the diffuser/confuser which is necessary to avoid the risk of cavitation

le and that cavitation phenomena do not occur. Assuming the transmission tube to assume almost the same diameter as the bellows and avoiding sharp angles between the transmission tube and the balloon by using a diffuser/confuser minimizes the risk of cavitation.

Empirically such a diffuser (Fig. 2) shall have a taper angle (α) of less than 7–8° if the Reynold number $Re = d(v)/\nu$ is high e.g. 5000–10 000, v being the flow velocity, d the diameter of the canal and ν the kinematic viscosity. The length of the diffuser (l_d) is related to the diameters of the neighboring channels by the equation

$$\frac{D-d}{2 \tan(\alpha/2)}$$

Equations (1) to (5) can be utilized to define the pressure losses in the system at the crucial moments when the influence of inertia or friction is maximal.

The pressure loss due to inertia (ΔP_i) is of great importance at the moment of reversal of flow when the volume flow acceleration is minimal and $\omega = 0$ (Fig. 3).

On the basis of Newton's first law $F = m \cdot a$ (force = mass \times acceleration times mass) and considering

$$\Delta P_i = \frac{1}{2} \rho \cdot l \cdot \omega^2$$

where ρ is the density of the fluid and l , the geometric length of the tube.

The minimal pressure difference $\Delta P_i = P_1 - P_2$ due to inertia is

$$\Delta P_i = -16 \cdot \rho \cdot V \cdot \omega^2 \left(\frac{l}{d_1^2} + \frac{l}{d_3^2} \right)$$

where ω is the frequency in cycles per second (Hz).

According to the angular frequency by $f = \omega/2\pi$ and l the lengths of the transmission tube and the

stem respectively. The location of the diameters d_1 and d_3 is shown in Fig. 1.

The pressure loss due to friction (ΔP_f) is maximal when flow velocity reaches its lowest value e.g. 90° after the loss due to inertia is maximal (Fig. 3). It is assumed that during the withdrawal phase of the cycle the total pressure in the intrauterine stem is equal to atmospheric pressure. This means that the pressure loss in the inlet holes in the intrauterine stem is negligible and that the pressure in the balloon is at least atmospheric. The most critical point is located at the border between the stem and the diffuser P_2 (Fig. 1). The diffuser gives a pressure recovery of about 70% of the dynamic pressure which increases the static pressure in the transmission tube.

The Bernoulli's equation gives

$$P_2 = P + \frac{\rho v^2}{2} + h$$

$$h = \lambda \frac{l \rho v^2}{2d}$$

$$\Delta P_f = \frac{32 \rho l v^2}{d_3^2} \left(1 + \lambda \frac{l}{d_3} \right)$$

$$\Delta P_f - P_2 - P_1$$

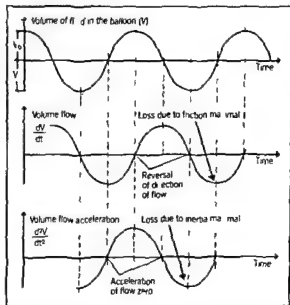


Fig. 3 Time relationship between the filling of the balloon, the volume flow and the volume flow acceleration. The critical points when pressure losses due to friction and to inertia are maximal are indicated.

Table I Units for mechanical excitation of the myometrium

	Unit no 1	Unit no 2	Unit no 3	Unit no 4
Oscillation volume ml	0.1-0.6	0.4-2.1	2-17	4-34
Length of transmission tube l_1 mm	300	300	300	<700
Diameter of transmission tube d_1 mm	6	10	70	73
Length of stem l mm	80	100	100	100
Diameter of stem d_2 mm	2.5	3.5	6	8.5
Length of diffusor l_4 mm	8	70	70	170

where v is velocity of the fluid, h the pressure loss in the stem and λ a friction factor which has the numerical value of about 0.02 for the tubes used in this system.

The pressure losses in the holes of the intrauterine stem must be minimized which is done by having as many holes as possible. The pressure difference ΔP_A over the holes between the inside of the stem and the intrauterine balloon is determined by the equation

$$\Delta P_A = \xi \frac{\rho v_A^2}{2}$$

where v_A is the fluid velocity in the holes. The coefficient of pressure loss ξ is 0.4 if the holes are poorly machined, e.g. have sharp edges. The value decreases to about 0.01 if the edges are rounded with a very small radius. The pressure loss in the perforations will be negligible if the total area of the holes is made to exceed the area of the intrauterine stem by a factor of 3-4 and if the machining is good.

It is evident that the frequency of the sinusoidal fluid movement is limited by the volume required to provide sufficient distension of the uterine wall and the relation between lengths and diameters of the transmission tube and the intrauterine stem. The smallest diameter of each unit is that of the stem which, with the empty balloon around it, has to be introduced through the cervical canal preferably without dilatation. A stem 7 mm in diameter can be used during pregnancy until the 14th week but about 4 mm outer diameter is the upper limit for non-pregnant uterus. The dimensions of the various components of four different units are given in Table I.

PERFORMANCE CHARACTERISTICS

The performance characteristics of the units were checked in an experimental set up using a large diameter water filled plastic basin as a uterine

dummy. The increase in static pressure as a result of the transport of fluid between pump and uterus was insignificant. Volumes of fluid close to the maximal capacity of each unit were oscillated and the resultant change in dynamic pressure was recorded. The results are given in Table II. One accepts the generation of a dynamic pressure ≤ 5 mmHg, unit number 4 should not be used at frequencies above 6 Hz. It made no difference to the results whether the rubber balloon enclosed the stem or not. This shows that properties of the wall of the balloon do not influence the recorded muscle response.

EVALUATION OF THE RECORDINGS

The measurements are made at distinct frequencies of mechanical excitation. The recordings are evaluated on the basis of the volume of fluid used for distension (V_{osc}), the frequency of distension (f) and changes in intrauterine pressure (ΔP_i). The uterine dimensions are determined from bimanual palpation in the frontal and sagittal planes and from sound measurement. The thickness of the uterine wall (h) is defined empirically as 1.0-1.25 cm (Table III). The uterine cavity during early pregnancy is considered to represent a hollow ellipsoid with the volume $V_i = \frac{4}{3}\pi r^2 b$. In this equation b is the sound measurement of the corpus cavity while $2(r+h)$ is the palpation measurement in the frontal and $2(r+h)$ the palpation measurement in the sagittal projection.

To obtain a quantity which will serve to characterize the functional state of the myometrium as well as the effect upon it of pharmacological and physical agents, a tonometric index of the myometrium is defined. For this one has to accept several approximations. The mechanical distension of the uterine muscle, for example, is taken to be the same as that the intraabdominal (extrauterine) pressure is

Table II Development of dynamic pressure mmHg in the high frequency range

	Frequency of oscillation Hz						
	10	9	8	7	6	4	2
x1 Osc vol 0.6 ml	3.5	3.0	2.0	<2	<2	<2	<2
x3 Osc vol 10 ml	6.0	5.0	4.0	3.0	2.0	<2	<2
x4 Osc vol 28 ml			8.0	6.0	4.5	2.0	0

aged during the measurement procedure. The free wall is furthermore considered to be incompressible, i.e. the volume of the uterine wall is the same before and at maximum distension.

In the mathematical model used to characterize the myometrium, the conditions in an imagined section through a rotation ellipsoid at the symmetrical rotation radius (r) are studied.

$$\left(\frac{3}{4\pi} \frac{V_1 + V_0}{b} \right)^{\frac{1}{3}}$$

Initially the contractile modulus (m), i.e. the ratio between the relative strain and the relative contraction of diameter is defined. The equation can have the following approximate expression provided the magnitude of the distension is small:

$$\frac{1}{r} \frac{r}{R} \left(1 - \frac{b}{B} \frac{r}{R} \right)$$

being $(r+h)$ and B ($b+h$).

As the second step the tangential stress (σ_t) in the innermost layer of the uterine wall is estimated in order to obtain equivalent expressions for pregnant and non-pregnant uteri. The imagined section of the rotation ellipsoid is treated as part of a cylinder.

$$P \left[\frac{R^2 + r^2}{R^2 - r^2} + P \frac{2R}{R^2 - r^2} \right] \frac{1}{R^2 - r^2}$$

The subscripts in the equation denote t tangential, i inner and u outer.

Table III Thickness of the uterine wall

Pregnancy after last menstrual period	cm
1st-3rd week	1.0
4th-10th week	1.25
11th-16th week	1.0

In order to eliminate the outer pressure the stress-difference $\Delta\sigma_t = \sigma_{ti} - \sigma_{to}$ is derived:

$$\Delta\sigma_t \approx \Delta P \frac{1 + (r/R)}{1 - (r/R)}$$

As the distension is small the differences between R_i and R_o , r_i and r_o , B_i and B_o and b_i and b_o are minute:

$$\frac{r_i + r_o}{R_i + R_o} \frac{b}{B} = \frac{rb}{RB}$$

Calculating the stress-difference in an axial direction (σ_x) in the inner layer of the same section through the ellipsoid gives the analogous expression:

$$\Delta\sigma_x \approx \Delta P \frac{(r/R)^2}{1 - (r/R)^2}$$

Obviously $\Delta\sigma$ (stress difference in the radial direction) equals $-\Delta P$.

The third step is to form an expression for the tangential strain ϵ_t . Differentiating the equation for the uterine volume:

$$V = \frac{3}{4} \pi r^2 b$$

gives the relation:

$$\frac{dV}{V} = 2 \frac{dr}{r} + \frac{db}{b}$$

Two cases can be viewed, namely:

1) $db/b=0$ and the difference in tangential strain $\Delta\epsilon_t = 0.5 (dV/V) = 0.5 (V_{\text{acc}}/V)$ when there is an extreme eccentricity of the rotation ellipsoid, i.e. it approximates a cylinder, and

2) $db/b=dr/r$ and $\Delta\epsilon_t = \frac{1}{2} (V_{\text{acc}}/V)$ when the eccentricity equals 1, i.e. the structure is sphere-like. The first relation is used for the non-pregnant condition and the second for the pregnant condition.

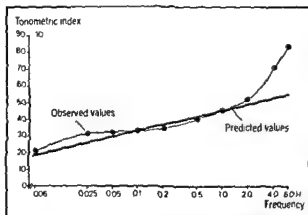


Fig 4 The row of frequency bound tonometric indices observed in one indomethacin treated pregnant woman together with the line of regression of the index values on log frequency. The equation $y = 45.1 + 12.3x$ is based on the observed indices for 0.05, 0.1, 0.2, 0.5, 1.0 and 2.0 Hz.

Finally Hook's law

$$\epsilon_i \approx \frac{1}{E} \left(\sigma_i - \frac{\sigma + \sigma_x}{m} \right)$$

provides the expression

$$E \approx \frac{\Delta \sigma_i - (1/m) \Delta \sigma_x - (1/m) \Delta \sigma}{\Delta \epsilon_i}$$

E is the proposed tonometric index of the myometrium. It must be emphasized that in this connection E 1) mirrors the difference between conditions before and at maximal distension, 2) is calculated from the appraisal of a section through the rotation ellipsoid assuming this to be part of a cylinder, 3) pays regard only to the stresses in the innermost layer of the myometrium where stresses are maximal.

It is evident from the results of the experimental study that one index value is characteristic only for a specified frequency of muscular excitation. A sequence of tonometric indices may therefore be plotted versus frequency in coordinate systems (Fig 4). When indices relating to one individual are used to evaluate myometrial effects of pharmacologically active agents, the line of the regression of the index values on the logarithm with base 10 of frequency is calculated utilizing the observations for frequencies within the range 0.05–2 Hz. Thereby the regression line is formed firstly for the individual $y = a + bx$ and secondly for a group of individuals according to the equation $y = b_1 + b_2 x_1 + b_3 x_2$ (Fig 5).

DISCUSSION

Three basic properties characterize the activity of smooth muscle: tonus, adaptability and rhythmicity. Various arrangements have been used for recording these qualities of the muscular wall of hollow organs in vivo. The results are generally difficult to comprehend, except for the dynamics of the thin wall of the urinary bladder. For this organ the convenience of monitoring slow alterations such as stress relaxation accompanying step-wise changes in volume has bridged the gap between conventional cystometry and the analysis of the elastic and viscoelastic properties of the bladder wall (2, 13, 14). In contrast, investigation of the mechanical behavior of the uterine wall in vivo has been hampered by the difficulty of using monitoring techniques that allow exact determination of the volume-pressure relationship (3, 4, 5, 6, 10, 11, 15). Development of the field of uterine dynamics might be promoted by the application in vivo of methods introduced in the study of specific aspects of the excitation-contraction coupling mechanism, such as the analysis of length-tension diagrams (8), force-velocity parameters (17) and stress response to sinusoidal strain (1).

It became obvious during earlier in vivo studies of the human uterus that the adaptation phenomena could be studied by step-wise distension of the organ or by the use of a slow, linearly increased stretch. However, data obtained from techniques applying oscillatory changes in length are easier to analyze. This is because sinusoidal movement can be realized with mechanical accuracy. A caveat remains in that the oscillations must be made at

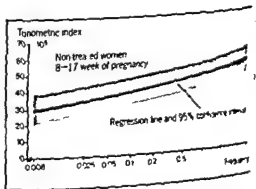


Fig 5 The line of regression of index values on log frequency for the group ($n = 9$) of non-treated pregnant women at 8–17 weeks of pregnancy. The control series $y = 47.4 + 8.2x$. The 95% confidence interval is symmetrically distributed around the regression line.

over a wide range of frequencies. Mechanical stimulation must be applied at a slow rate if one is to analyze the relationship in phase between distension and development of tension. High frequencies are needed in order to study vibration induced relaxation (13). With the present device there are problems in ascertaining the exactness of operation at the low (<0.01 Hz) as well as in the high (>4 Hz) frequency ranges but for different reasons. Mechanical instability hinders the function of the bellows in the low range while hydrodynamic complications render the pressure measurement unreliable at high frequencies. At present these factors impede study of for example the effect upon the uterus of cervix of mechanical influences causing fast effect (18).

In the present investigation the response of the uterine wall to mechanical and to pharmacological stimulation has been recorded primarily as a variation in intrauterine pressure. It should be emphasized that the tonometric index defined with this experimental model and suggested as a measure of effect is to be regarded as a synthetic discriminant for which although it has the dimension of an elasticity module (stress/strain) does not imply any single physical property of the uterine wall.

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THE USE OF SALBUTAMOL IN OBSTETRICS

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Sympathomimetic amines have been used with aim of abolishing uterine contractions. On the basis of this from an in vivo technique for testing the specificity of the beta₂-receptor stimulating agents salbutamol was used for a clinical trial. Five case histories were selected in order to illustrate the possibility of utilizing salbutamol in emergency cases with the aim of achieving uterine relaxation during the period of time between admission of the patient and the commencement of delivery by for example caesarean section.

The efficacy with which beta receptor adrenergic drugs induce relaxation of smooth muscle has been documented and a wealth of literature is available on this matter (4, 9, 2). A serious adverse side effect however has been the influence of the drugs on the beta₁ receptor mediated properties of cardiac function especially the production of tachycardia. In the aim of avoiding this reaction which is sometimes very unpleasant for the patient new drugs have been developed during the last few years. These drugs are characterized by more pronounced specificity for the beta₂ receptors in smooth muscle (5, 3, 1). Measurement of the relaxant effect upon the pregnant human uterus in vivo has indicated that the ratio between the effects on the uterus and on heart was especially favorable for some of the new drugs e.g. salbutamol and fenoterol. In clinical trials salbutamol was also shown to postpone delivery in a high proportion of patients with premature contractions (8). The availability of agents capable of relaxing the uterine smooth muscle would however be advantageous in cases of complications during labor at full term. In this report the results of the treatment of five cases with salbutamol will be reported.

METHODS

Pregnant women presenting themselves in emergency situations during labor were given salbutamol with the aim of inducing uterine relaxation. Twenty five mg of the drug were added to 1000 ml 5% dextrose or 0.9% sodium chloride. The infusion set using a Univac drip counter was connected to an indwelling catheter in the brachial vein. Maternal systolic and diastolic blood pressures and heart rate were determined at intervals no longer than 15 min. The fetal heart rate and the uterine contractions were recorded continuously with a Hewlett Packard or Corometrics cardiotocograph. The dosage of salbutamol was individually adjusted depending on maternal cardiovascular function and uterine activity within the range from 2.5 µg/min to 75 µg/min usually 7.5-22.5 µg/min.

RESULTS

The results are given in the form of case histories. Among a large series of patients studied 5 cases were selected for this presentation. Data in general are given in Table I.

Case 1 A M 27 years III gravida II para. Admitted to the hospital at full term active labor for 10 h. Temperature 39.0°C cervix 6 cm dilated membranes ruptured fetus in a transverse lie one shoulder impacted in the pelvic inlet the arm prolapsed. Duration of contractions 45 sec intervals 2-3 min. FHR record disclosed variable and late decelerations. Caesarean section was decided upon. With the aim of achieving uterine relaxation and relieving fetal asphyxia the presence of which was made highly probable by the late deceleration phenomena a salbutamol infusion was started at a dose of 17.5 µg/min and increased to 37.5 µg/min. The intervals between contractions increased and their amplitude decreased. The maternal heart rate rose from 100 beats/min to 170 beats/min which necessitated a reduction of the dose of salbutamol. Changes in systolic and diastolic blood pressure were minimal. The infusion of salbutamol was continued until the baby was delivered through a lower segment incision. The uterus was well relaxed and the infant easily extracted. Birth weight was 3.8 kg. Apgar score 7 at 1 min and 10 at 5 min.

Table 1 Details of patients

Patient	Age	Gravity	Parity	History	Remarks
1 A M	22	III	II	2 normal deliveries 1 infant dead after 7 months	Transverse lie
2 M T	20	II	0	1 abortion	Twin conception req
3 S H	23	IV	II	1 normal delivery 1 stillborn	Abruptio placentae fetal distress
4 R W	27	II	I	1 abortion	Fetal distress
5 M N	29	III	II	1 normal delivery	37 week premature contractions
				2 caesarean sections cephalopelvic disproportion	

Case 2 M T 20 years II gravida 0 para Admitted in active labor with an undiagnosed twin pregnancy gestational age 37 weeks The first twin was delivered normally birth weight 1.5 kg Apgar score 10 at 10 min The second twin presented with an incomplete foot presentation The cord prolapsed the pulse rate in the cord was counted to be 60 beats/min Clinical signs indicated an abruption of the placenta Myometrial tension increased and a constriction ring formed at the junction between the upper and the lower segment of the uterus around the knee of the infant which was completely stuck Pure oxygen was given by mask and an i.v. infusion of salbutamol was started at a dose of 75 μ g/min The constriction ring relaxed within 2 min after the initiation of the infusion and the fetus was easily extracted Birth weight 1.7 kg Apgar score 4 at 1 min and 7 at 10 min

Case 3 S H 23 years IV gravida II para A.m.v. during active labor membranes ruptured The amniotic fluid was thick and meconium stained The cervix was similar to that of a 36-week pregnancy The fetus was in vertex position at station 0 The uterus was hard as cervix 100% effaced uterine os 3 cm dilated The fetal heart rate base line showed reduced beat-to-beat variability Early deceleration change was present with a reduction in heart rate amounting to more than 60 beats/min Change in maternal position together with enox administration had no influence on the FHR then salbutamol infusion was started at a dose of 25 μ g/min The contractions stopped completely after 11 min FHR became normal with reappearance of beat-to-beat variation (Fig. 1) A caesarean section was performed and infant with a birth weight of 2.7 kg was delivered 49

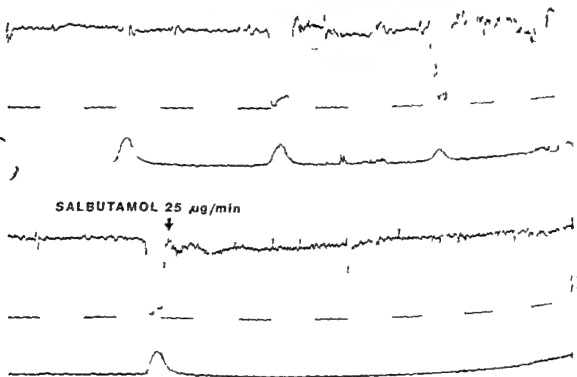


Fig. 1 Salbutamol infusion in a case with abruptio placentae Deceleration and reduced beat-to-beat variation

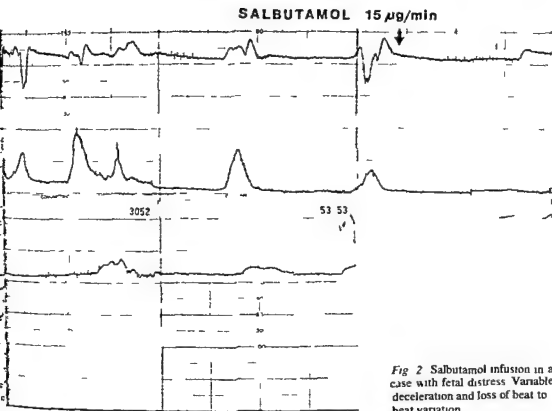


Fig 2 Salbutamol infusion in a case with fetal distress. Variable deceleration and loss of beat to beat variation

at 3 at 1 min and 7 at 5 min. The placenta showed an area of one third of its area.

Case 4 R W 27 years II gravida I para. Admitted with prolonged labor membranes ruptured. FHR showed beat to beat variability and so-called variable decelerations occurred (Fig 2). A caesarean section was started. A salbutamol infusion in the dosage of 15 µg/min was started. Uterine contractions ceased completely after 3 min. The FHR pattern which was monitored up to delivery continued however to show loss of beat to beat variation. Birth weight 4.1 kg. Apgar score 6 at 1 min and 10 at 7 min.

Case 5 M N 29 years III gravida II para. Admitted at the 7th week of pregnancy because of premature labor contractions which were regular. Cervix was not dilated. Uterine os closed. Two earlier caesarean sections due to cephalopelvic disproportion. A salbutamol infusion was started at a dose of 15 µg/min and increased to 25 µg/min after 15 min. Uterine contractions disappeared completely. Salbutamol was continued with oral administration at a dose of 7 mg x 4. The pregnancy continued untroubled.

DISCUSSION

The sympathetic action of adrenergic agonists is characterized in terms of alpha and beta receptor mediated responses each possessing different receptors. The beta receptors are further subdivided

into beta₁ receptors within the heart with chronotropic and inotropic effects and beta₂ receptors in smooth muscles with relaxing properties. In addition the release of cyclic AMP induces general effects on carbohydrate and lipid metabolism.

Even though adrenergic agonists employed with the aim of arresting uterine contractions have been free from alpha receptor stimulating properties they have had a mixed beta₁ and beta₂ receptor affinity. The widening of pulse amplitude and sometimes a resultant decrease in mean blood pressure were not infrequently severe enough to prevent further use of the drug. Efforts were made to abolish the side effects by the use of simultaneously administered beta receptor blocking agents. However such an action was neither supported by theoretical considerations nor was it sufficiently successful in its practical application.

Pharmacological research made possible the synthesis of agents with more selective beta₂ receptor affinity and several such agents have been marketed. In the literature reports are available presenting results of experiments regarding the

Table I Details of patients

Patient	Age	Gravity	Parity	History	Remarks
1 A M	22	III	II	2 normal deliveries 1 infant dead after 7 months	Transverse lie
2 M T	20	II	0	1 abortion	Twin constriction ring
3 S H	23	IV	II	1 normal delivery 1 stillborn 1 abortion	Abruptio placenta foetal distress
4 R W	22	II	I	1 normal delivery	Fetal distress
5 M N	29	III	II	2 caesarean sections cephalopelvic disproportion	37 week premature contractions

Case 2 M T 20 years II gravida 0 para Admitted in active labor with an undiagnosed twin pregnancy gestational age 37 weeks The first twin was delivered normally birth weight 1.5 kg Apgar score 10 at 10 min The second twin presented with an incomplete foot presentation The cord prolapsed the pulse rate in the cord was counted to be 60 beats/min Clinical signs indicated an abruption of the placenta Myometrial tension increased and a constriction ring formed at the junction between the upper and the lower segment of the uterus around the knee of the infant which was completely stuck Pure oxygen was given by mask and an i.v. infusion of salbutamol was started at a dose of 75 µg/min The constriction ring relaxed within 2 min after the initiation of the infusion and the fetus was easily extracted Birth weight 1.7 kg Apgar score 4 at 1 min and 7 at 10 min

Case 3 S H 23 years IV gravida II para Admitted during active labor membranes ruptured The amniotic fluid was thick and meconium stained The cervix was similar to that of a 36-week pregnancy The fetus was in vertex position at station 0 The uterus was firm cervix 100% effaced uterine os 3 cm dilated Fetal heart rate base line showed reduced beat-to-beat variability Early deceleration change was present with a reduction in heart rate amounting to more than 60 beats/min Change in maternal position together with oxytocin administration had no influence on the FHR pattern salbutamol infusion was started at a dose of 75 µg/min The contractions stopped completely after 11 min Fetal heart rate became normal with reappearance of beat-to-beat variation (Fig. 1) A caesarean section was performed and a 3.5 kg infant with a birth weight of 3.5 kg was delivered

SALBUTAMOL 25 µg/min

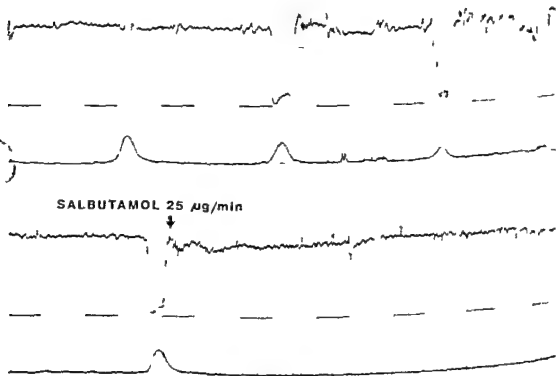


Fig. 1 Salbutamol infusion in a case with abruptio placentae Deceleration and reduced beat-to-beat variation

STUDIES ON THE MODE OF ACTION OF CLOMIPHENE CITRATE

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Abstract In order to further investigate the mode of action of clomiphene citrate 5 oophorectomized women in the age range 28 to 41 years were followed with weekly determinations of serum FSH and LH for 20 weeks after oophorectomy. They received no treatment during the first 4 weeks but during the next 16 weeks they were given estradiol 2 mg \times 7 daily orally and from the 12th to the 16th week in addition clomiphene citrate 50 mg \times 2 daily. The high level of FSH after the oophorectomy decreased gradually during the treatment with estradiol alone but in effect was partly neutralized by clomiphene citrate as it observed a significant increase in FSH within 1 week of initiating treatment with clomiphene citrate. On the other hand we did not find any significant changes in FSH when 4 weeks after treatment with clomiphene citrate was discontinued. The high level of LH after oophorectomy did not show any significant changes during the whole treatment period. It is concluded that clomiphene citrate primarily stimulates FSH secretion most probably in competition with estradiol for the receptor sites in the hypothalamus or the pituitary. In addition it is concluded that clomiphene citrate has a prolonged effect probably because of a tight binding to the receptor sites.

Greenblatt (6) first observed that clomiphene citrate could induce ovulation in a high percentage of anovulatory women. This treatment has been widely used for induction of ovulation in sterile women (3, 8, 13, 14, 15, 16, 26). Nevertheless the mode of action of clomiphene citrate on the human reproductive system is still not quite clarified.

Two principal theories concerning the action of clomiphene citrate have been proposed. The first theory claims that clomiphene citrate acts on the hypothalamic-pituitary system. It is believed that clomiphene citrate or rather the *cis* isomer of the molecule (7, 29) blocks the receptor sites in the hypothalamus or the pituitary in competition with estradiol thus causing an increased gonadotropin

secretion (11, 21). This theory is in agreement with the investigation by Eisenfeld & Axelrod (5) and Roy et al (22) who found a decreased ³H-estradiol uptake in the hypothalamus and the pituitary in rats treated with clomiphene citrate compared with control animals.

The second theory indicates a direct action of clomiphene citrate on the biosynthesis of steroids in for instance the ovary and the placenta. Some investigators (9, 24, 25) have observed that clomiphene citrate causes an increased conversion of testosterone to estradiol in placenta microsomes *in vitro*. This effect was due to an inhibition of the nicotinamide adenine dinucleotide phosphate (NADPH) cytochrome c-oxidoreductase system with a subsequent availability of NADPH which is an obligatory cofactor for aromatization of the A ring. Other investigators have in *in vitro* experiments shown that clomiphene citrate stimulates the 3 β -ol-dehydrogenase Δ^5 isomerase in human corpora lutea slices (10) and 17 β -ol dehydrogenase in fungus homogenates (1). Both enzymes together with NADPH are necessary for the steroid transformation and it appears from these *in vitro* investigations that clomiphene citrate stimulates important steps in the estrogen biosynthesis.

The aim of the present study was further to elucidate the effect of clomiphene citrate on the hypothalamic-pituitary system.

MATERIAL AND METHODS

The material consists of 5 women aged 28 to 41 who were bilaterally oophorectomized because of various gynecological diseases but it should be noted that none of the women showed any signs of estrogen deficiency before

pharmacological properties and the order of precedence of specificity of one and the same agent for the β_1 and β_2 receptors respectively. The effect on bronchial muscle is quantitatively evaluated from in vitro and in vivo experiments while the action on uterine smooth muscle most often has been described from in vitro studies only. Recently an in vivo technique for the quantitative evaluation of the effect upon the pregnant uterus has been presented (6). It was demonstrated that salbutamol and feneterol were the most potent agents among the β_2 receptor stimulating agents with respect to uterine relaxation.

In all of the studied cases salbutamol had a dramatic effect on uterine activity. Most often uterine contractions were completely abolished, this even in cases with ruptured membranes and fully dilated cervixes. The influence upon the resting pressure could not be determined because only external tocometry was employed. The influence upon maternal systolic blood pressure was negligible while the diastolic pressure usually decreased sometimes by as much as 25–50%. The influence upon diastolic pressure was however both dose dependent and subjected to pronounced interindividual variation. The same was true regarding the effects on maternal heart rate.

The influence upon FHR of infusions of salbutamol to the mother was inconsistent; most often changes in heart rate could not be detected. The late deceleration pattern, accepted as a sign of fetal asphyxia, is evidently not provoked as soon as intrauterine contractions are inhibited. The disappearance of the late deceleration pattern can therefore not be utilized as a parameter of change in fetal heart rate. The fact that beat-to-beat variations, independent of uterine activity, can be suggested as a more useful parameter for the study of the effect of salbutamol on the oxygen supply to the fetus. This suggestion is valid provided analgesic drugs have not been administered to the mother in large quantities. This series did not include enough patients to allow an analysis of the reappearance of beat-to-beat variation following the administration of salbutamol.

In the literature data have accumulated about the beneficial effect upon the fetus of an infusion to the mother of sympathomimetic amines. Experimental work with subhuman primates has indicated that the infusion of isoproterenol, which is a β_1 and β_2 receptor stimulating agent, causes a decrease

in arterial fetal PO_2 (7). This finding might be due to the fact that maternal cardiovascular function was affected to a major degree but it might also be ascribed to the situation of using animals under anaesthesia for the investigation. This criticism makes it uncertain whether the results are applicable in human clinical obstetrics. Besides these explanations offered above, there are reasons to believe that the relaxation of smooth muscle with an increased conductance in some vascular channels can produce a partial redistribution of blood flow from the visceral to the systemic circulation. Further studies are necessary and have been initiated for the elucidation of these mechanisms in humans.

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trate in the above mentioned doses. Within one week the mean value of serum FSH had increased from 100 mIU/ml to 145 mIU/ml and this level of FSH was maintained during the following 3 weeks. The increase in serum FSH was significant ($P < 0.01$). It should, however, be noted that the high treatment level of FSH was never reached. After treatment with clomiphene citrate was discontinued a slight decrease in serum FSH was seen but this fall was not significant within the 4 weeks of observation.

Fig. 2 shows in the same way the changes in the serum LH level in the 5 women during the 20 weeks of observation. It appears that 2 weeks after the oophorectomy the serum LH values are within the range for postmenopausal women and the serum FSH level did not show any significant changes during the remaining part of the observation period. Clinically it was characteristic of all 5 women that they had climacteric complaints (hot flushes, sweating, headache) within the first 4 weeks after the oophorectomy. These symptoms disappeared completely during the treatment with estradiol alone but within one week after treatment with clomiphene citrate was initiated all 5 women again had the same complaints to a certain degree and the symptoms increased during the next 3 weeks. After treatment with clomiphene citrate was discontinued the climacteric complaints decreased very slowly and one out of the 5 women had still symptoms 4 weeks after the last tablet of clomiphene citrate had been taken.

DISCUSSION

The present investigation confirms that clomiphene citrate undoubtedly has a central effect on the hypothalamic-pituitary system. It seems as if clomiphene citrate primarily stimulated the secretion of FSH whereas we did not observe any significant stimulation of the LH secretion as reported by several other investigators (11, 13, 19, 28, 29). It should, however, be noted that one out of 5 women in our study showed a significant increase in both serum FSH and LH. The observed variation in the LH response to clomiphene citrate might be explained by 1) different preparations and doses of the oestrogens used, 2) the presence or absence of oestrogens in the women investigated and 3) different duration of the treatment with clomiphene citrate. The level of oestrogens seems to be of decisive

importance for the gonadotropin response to treatment with clomiphene citrate. Thompson & Melinger (28) showed in a patient with Turner's syndrome who did not receive any substitution therapy with oestrogens that clomiphene citrate did not alter the urinary excretion of FSH and LH at all. The same patient was then treated with diethylstilbestrol until the excretion of gonadotropins was significantly suppressed and repeated stimulation with clomiphene citrate during continued treatment with oestrogen now caused a significant increase in the excretion of both FSH and LH. They concluded therefore that a certain oestrogen level is necessary for clomiphene citrate to stimulate the secretion of gonadotropins. On the other hand, Thompson et al. (27) found a marked suppressive effect of estradiol benzoate on the LH response to exogenous luteinizing hormone releasing hormone when the concentration of estradiol was in the range of 400–1000 pg/ml serum and a similar suppression was observed by Pedersen et al. (30) when the patients received a daily dose of 2 mg estradiol + 1 mg oestril.

Another important observation is that clomiphene citrate seems to exert an effect for several weeks as we did not observe any significant decrease in the FSH level within the first 4 weeks after the treatment with clomiphene citrate was discontinued. Our finding is in good agreement with the observation reported by Schreiber et al. (23) who after administration of ^{14}C labelled clomiphene citrate found that even if 51% of this drug was eliminated within 5 days they could still detect small amounts of ^{14}C labelled clomiphene citrate in the patients 6 weeks later. This prolonged effect is probably due to a tight binding of clomiphene citrate to the receptor sites in the hypothalamus or the pituitary.

Furthermore we would like to add that our observations indicate that FSH and LH in women to a wide extent are regulated independent of each other. Since only one gonadotropin releasing hormone has been found so far the mechanism of this regulation is at present obscure.

Several *in vitro* experiments (1, 9, 10, 24, 25) indicate that clomiphene citrate in addition to the central effect on the hypothalamic-pituitary system also might have an effect directly on the ovary but a similar effect *in vivo* has not yet been found.

Crooke et al. (4) have however in 6 amenorrhoeic women observed that clomiphene citrate aug-

mented the effect of exogenous FSH by a factor of 173% as shown by the excretion of estrogens. This finding might be explained by a direct effect of clomiphene citrate on the ovary.

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THE BEHAVIOUR OF MICROSOMAL MONO ELECTRON CARRIERS IN HUMAN PLACENTA DURING LATE PREGNANCY

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Abstract The activities and the content of enzymes in mixed function oxidase systems (cytochromes $P-450$ and b_5 NADPH-cytochrome c reductase and ADH cytochrome b reductase) in microsomes from 114 human placentae were found to increase from the 34th up to the 38th-39th week of gestation and later to decrease until the 43rd week. Such a process can be related to the following: (i) the phospholipid content of microsomal membranes (ii) the protein synthesis possibly induced by steroids such as androstenedione and testosterone.

The function currently assigned to the placenta as a highly active organ suggests that it might metabolize foreign compounds introduced by the mother thus acting as a protective barrier for the fetus or at least as a substitute organ for the metabolism of such compounds with respect to both maternal and fetal liver. During the transport from maternal to fetal circulation foreign compounds such as drugs and chemical additives cross the placenta simply by diffusion (11).

The enzymatic systems in liver and placenta metabolizing most foreign compounds are termed mixed function oxidases (27) or monooxygenases (28) and consist of cytochrome $P-450$ which is the terminal oxidase, another hemoprotein (cytochrome b_5) and two flavoproteins (NADPH cytochrome c reductase and NADH cytochrome b_5 reductase). The occurrence of cytochrome $P-450$ in human placenta was reported by Meigs and Ryan (29) at term and by Juchau et al (17) in the early weeks of gestation. This hemoprotein is mostly found in syncytiotrophoblast together with cytochrome b_5 and the flavoprotein NADPH-cytochrome c reductase (32). As for rat live microsomes cytochrome $P-450$ spectra with different

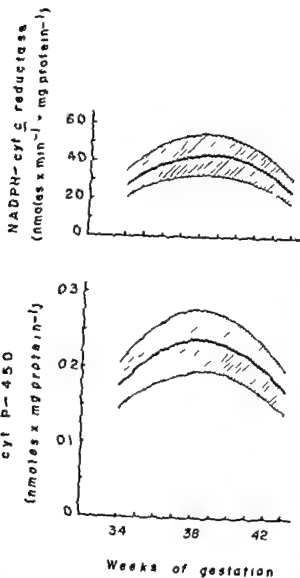
substrates and ligands have been obtained in microsomes of human placenta and some differences in this respect between the two types of microsomal preparations have been described (17-32).

The lack of data concerning the activities and content of enzymes of the mixed function oxidase systems in the course of gestation has prompted us to investigate this subject in order to determine whether the metabolizing capacity of the placenta with respect to some drugs changes according to the time of gestation. The results show increasing content and activity of these enzymes from the 34th until the 38th-39th week of gestation and after that a decline from the 39th to the 43rd week.

MATERIALS AND METHODS

The microsomal fraction was obtained according to Omura & Sato (35) from 114 human placentae between the 34th and the 43rd week of gestation after spontaneous delivery or Cesarean section. The placentae were immediately taken and poured into 0.15 M KCl containing 50 mM Tris HCl buffer pH 7.5 at 0-4°C after removal of membranes and umbilical cord. The microsomal fraction was suspended in the medium described above yielding a protein concentration of about 20 mg/ml. Proteins were determined by the biuret method (24).

Difference absorption spectra were obtained in a dual wavelength/split beam Aminco Chance spectrophotometer. Cytochrome b was measured from the NADPH difference spectrum of the CO-treated microsomes using $\Delta\epsilon$ (474-409 nm) = 165 cm² mM⁻¹ (10). Cytochrome $P-450$ was measured from the NADPH plus dithionite difference spectrum of CO treated microsomes using $\Delta\epsilon$ (450-490 nm) = 91 cm² mM⁻¹ (22). This method required base line determination with both cuvettes already saturated with CO which allowed us to determine cytochrome $P-450$ irrespective of hemoglobin contamination. Because of peculiar spongy structure placenta is quite rich in



1 Kinetics of microsomal cytochrome P-450 and NADPH-cytochrome c reductase of human placenta. The cytochrome content and enzyme activity have been estimated at different stages of pregnancy in the microsomal fractions as described under Materials and Methods. Placentae were tested weekly from the 34th to the 43rd week of gestation and each value is the mean of 7-14 experiments. The curves were obtained from a 11 degree polynomial correlation by the least squares method.

and hemoglobin can be hardly removed during microsomal isolation. NADPH cytochrome c reductase was measured following cytochrome c reduction at 446 nm in the presence of NADPH (47). NADPH-cytochrome b₅ reductase was measured following NADH oxidation at 340 nm in the presence of potassium ferricyanide as electron acceptor (38). A Honeywell computer was used for data processing.

RESULTS

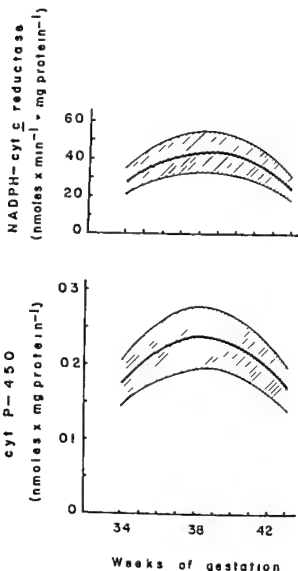
All the four enzymes were found to increase from the 34th to the 38th week of gestation and to decrease from the 38th-39th to the 43rd week of gestation (Figs 1 and 2).

The levels of microsomal enzymes as a function of time result from a 11 degree correlation according to the minimum square method.

DISCUSSION

The most striking feature is the similarity between the pattern of enzyme activities and cytochrome contents. The fact that the four enzymes in the placenta behave similarly is a further evidence of the hypothesis that they are closely related and indispensable for oxidation reactions at microsomal level as suggested by Hildebrandt & Estabrook (14) and by Mannering et al (76) for liver tissue.

As the placenta goes rapidly towards maturity and ageing, it can be assumed that the time course of the four enzymes biochemically reflects what is currently termed 'placental ageing'. Several studies have emphasized an increase in microsomal oxidative enzymes and a variation of oxidative capacities with respect to drugs in rat liver during the early stages of growth of the animal (7, 18, 20, 37). On the other hand a decrease in NADPH cytochrome c reductase activity as well as in the cytochrome P-450 content was found to occur in old rats as compared to young rats (12, 21). Such changes could be related to modifications in the phospholipid content of membrane structures. Experiments *in vitro* have also demonstrated that phospholipase D brings about an inactivation of liver microsomal NADPH cytochrome c reductase (25). Furthermore phospholipid content of rabbit placenta has been found to increase as gestation proceeds (1). There are two possible causes for the variation in phospholipid content of human placenta during gestation: first, postulated (i) changes in endogenous phospholipase activity (ii) changes in the extent of lipid oxidation. It has been demonstrated that there is a decrease in respiratory control in aged rat liver mitochondria is partly related to the activation of endogenous phospholipases (76). The presence of phospholipases was also demonstrated at the normal level (33). Moreover it has been postulated that an increase in lipid peroxidation could result



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HCT AND THYROID FUNCTION IN MOLAR PREGNANCY

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HCT TSH T₃ T₄ PBI T₃RSU TBG binding capacity BMR and thyroid uptake of radioiodine were measured simultaneously on the volunteers with molar and normal pregnancy. TRH stimulation tests were performed on the two groups. The serum TSH levels were higher in association with molar pregnancy than in normal pregnancy. T₃ T₄ PBI and thyroid uptake of radioiodine suggested hyperfunction in molar pregnancy with a greater increase than in normal pregnancy. TBG binding capacity T₃RSU were similar in the two groups. The results of TRH stimulation tests ranged widely in both molar pregnancies but were within the normal range in the subjects with normal pregnancy. These findings suggest thyroid hyperfunction in the molar pregnancy is due to a larger amount of HCT than in normal pregnancy.

It was suggested in 1955 that a thyroid stimulating substance may exist in the human placenta and a purified protein with TSH activity was isolated by Hennen et al (2). Hershman et al (3) isolated a highly purified glycoprotein with TSH activity from human term placenta by the method of Hershman et al (3). This protein hormone is human chorionic thyrotropin (HCT) and we studied on its biological, biochemical and immunological characters in 1972 (4, 5). We demonstrated that the serum HCT level estimated with radioimmunoassay using ¹²⁵I-HCT and anti-HCT serum increased progressively during pregnancy (6) and that the progressive elevation of thyroid function during pregnancy was due not to the increase in thyroid function, but to HCT originating from the placenta.

Sharma (8), Singh (9) and Dowling (10) described changes of the serum protein binding iodine and thyroid binding capacity (TBG binding capacity) thyroid uptake of radioiodine and basal metabolic rate in patients with the molar pregnancy

and found them similar to those in women with normal pregnancy but to a more extreme degree.

Odell et al (11) using bioassay detected high TSH activity in the serum of patients with molar pregnancies. Hershman et al extracted a thyroid stimulator from the chorionic tumour tissue and the serum of a patient with coexistent hyperthyroidism and hydatidiform mole; this they called molar thyrotropin (12).

Recently we have detected large amounts of HCT in the serum of patients with molar pregnancies by radioimmunoassay using ¹²⁵I-HCT and anti-HCT serum (6) and suggested that the HCT level was a useful indicator in the treatment of chorionic neoplasm (13).

In this paper the serum HCT levels and pituitary thyroid function in patients with hydatidiform mole and those with normal pregnancy are compared.

SUBJECTS AND INVESTIGATIONS

HCT, thyroid stimulating hormone (TSH), triiodothyronine (T₃), thyroxine (T₄), PBI, effective thyroxine ratio (ETR), ¹²⁵I-triiodothyronine resin sponge uptake (T₃-RSU), TBG binding capacity, basal metabolic rate (BMR) and thyroid uptake of radioiodine were measured simultaneously in 30 patients with hydatidiform mole and 40 normal pregnant women at an early stage of gestation (weeks 8-15). Thyrotropin-releasing hormone (TRH) stimulation tests were performed in 30 patients with hydatidiform mole and in 20 women in early pregnancy.

The serum HCT level was determined by a radioimmunoassay using ¹²⁵I-HCT and anti-HCT serum reported previously (6). The serum TSH level and the serum T₃ level were determined by radioimmunoassay using kits of Daisaku Radioisotope Laboratories (Tokyo, Japan). The serum T₄ and ETR were measured with Rev-O-Mat T₄ kit and Rev-O-Mat ETR kit of Mallinckrodt Corporation (USA). The serum PBI level was determined with an autoanalyzer technique and PMR was determined with the

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TUBAL STERILIZATION

*With Special Reference to Electrocoagulation through
the Laparoscope*

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In the past 5 years 1 168 tubal sterilizations have been performed. One thousand and twelve of these were performed by dividing the tube by diathermy through the laparoscope. Since the first cases and with increasing experience complications have been infrequent at the time of a slight bleeding from the mesosalpinx. So far we have had ten failures probably due to lack of experience. In every failure it was found at re-examination that the tube had not been divided—at least at one side. The procedure is quick and simple. The patient suffers little inconvenience, has a very small scar and is in hospital only a short time. It has been used in post partum cases but is not suitable for patients who have had several abdominal operations.

The frequency of legal abortions has increased in many countries in recent years. Unwanted pregnancy appears to be more common than before in spite of improved methods of contraception. Many couples feel that older methods of contraception are no longer acceptable once they have experienced the simplicity of the pill. On the other hand many women are able to take the pill only for a limited time and others have been scared by reports of various complications. The intra uterine contraceptive devices also produce side effects. They have a relatively low acceptability rate. A practical alternative for many couples will then be sterilization of one partner. When they have all their children they want and have decided on permanent contraception sterilization may be considered. Many methods have been employed in the past for the purpose of tubal sterilization. The Madlener operation (7) or the modification of Pomeroy is probably the most frequently accepted method. Other possibilities are total salpingectomy. Al-

drige's temporary procedure and cornual excision of the uterus.

Post partum sterilization has been the method of choice in many hospitals. A small incision gives ready access to the tube 2-3 days post partum. As early as 1932 Skajaa (11) reported the first series of 126 cases of post partum sterilization from Oslo University Hospital. In 1939 Amundsen (1) presented an additional 150 cases. Blom Hartvigsen (4) carried out a survey of 267 new cases from the same hospital in 1951. The combined series of 543 cases were complicated by 18 cases of thrombosis and 1 severe infection. The mortality rate was 0.18%. No failures were mentioned. In 1957 Jensen & Lester (5) published a series of 734 cases of sterilization by the Madlener operation from Denmark. There were two deaths but no other major complications.

A sterilization method should be easy with minimal risk to the patient. It should be as effective as possible and should not carry any side effects. In 1937 Anderson (2) suggested the possibility of tubal cauterization during peritoneoscopy. Power & Barnes (10) in Michigan had the same idea but none of them have published reports of patients treated in this way.

With improved equipment and the fiberoptic light laparoscopy has gained in popularity in recent years. Palmer (8) and Steptoe (12) introduced a new improved laparoscope with fiberoptic light and surgical equipment for different procedures. A simple method of tubal electrocoagulation under laparoscopic control was presented.

Following the initial presentations by Palmer and by Steptoe many investigators have used their methods unaltered or partly modified and several

Table I *Survey of the series*

	No. of patients					
	1970	1971	1972	1973	1974	Total
Resect Pomeroy	73	40	19	14	10	156
Palmer	76	175	186	258	317	1 012
Total	149	215	205	272	327	1 168

series have been published. Steptoe had one failure in 310 cases. No complications were described. Svennerud & Åstedt (13) in Sweden presented a series of 42 cases in 1968. Listen et al. (6) published a series of 760 patients sterilized by laparoscopy. Nine became pregnant after the operation, but only 4 were found to be operative failures. Six patients required laparotomy to control bleeding.

Cohen, Taylor & Kass (3) reported a series of 50 cases treated with a slightly different technique which allowed histological verification of tubal tissue. They had no failures and no complications followed.

Peterson & Behrman (9) recently published a series of 186 women sterilized by electrocoagulation and cutting of the tubes. One pregnancy occurred while 5 patients required laparotomy to control bleeding. Perforation of the stomach occurred once and in 5 patients the uterus was perforated by the instrument inserted to manipulate the organ.

Wheless (14) from experience in 75 patients advocated the method as an excellent procedure for sterilization in the out-patient department. In his series hemorrhage occurred in 3 patients, one of whom required laparotomy. One patient had post-operative aspiration pneumonia. The observation time was too short to decide on failures.

Pomeroy's tubal resection has previously been the method of choice for sterilization in our department. During the last 5 years, however, this method

Table II *Age distribution*

Age	Number
20-24	30
25-29	189
30-34	335
35-39	384
40-44	212
45-49	18
Total	1 168

Table III *Tubal sterilization. Time of operation*

	At legal abortion	1-5 post partum day	Intra-uterine operation	Total
Resect Pomeroy	63	59	34	156
Palmer	487	113	41*	1 041
Total	550	172	446	1 168

has gradually been replaced by tubal electrocoagulation under laparoscopic control. In patients who have had a number of laparotomies and extensive adhesions can be expected, we still perform laparotomy if we do not succeed at once.

MATERIAL AND METHODS

The present paper deals with a series of 1168 patients sterilized at the Department of Gynecology, Akershus Hospital from 1970 through 1974. Of these 116 were treated

Table IV *Medical indications for sterilization*

Psychoneurosis	19
Section caesarea 2-4 times previously	13
Pre-eclampsia	6
Polyarthritis	4
Osteochondrosis columnae	3
Mb. Bechterew	2
Ischias	2
Pelvic relaxation	2
Rhesus immunization	1
Tuberculosis	1
Lupus erythem. Diss.	1
Boeck's sarcoid	1
Hyperthyreosis	1
Hypothyreosis	1
Diabetes mellitus	1
Morbus reus	1
Hypertension	1
Asthma bronchiale	1
Hemophili carrier	1
Ca. cerv. ut. st. 0	1
Chondrodystrophia	1
Colitis ulcerosa	1
Cephalalgia atypical EEG	1
Seq. Poliomyelitis	1
Subileus	1
Hab. postpartum bleeding	1
Cancer mammae op.	1
Glaucoma	1
Prolapsus gen. op.	1
Hernia diaphragmauca	1
Paresis	1
Mb. cordis	1
Epilepsia	1
Abortus habitus	1
Hyperemesis	1
Advanced age	1
Earlier malformed children	1

Table V Deliveries prior to sterilization

Children	1970	1971	1972	1973	1974	Total
None	8	4	3	17	4	36
1	40	76	74	124	158	472
2	90	16	10	116	151	603
3 or more	11	9	8	15	14	57
Total	149	215	205	272	327	1168

Verres method while 1012 were treated by electrocoagulation during laparoscopy (Table I). The table also shows the increasing tendency to prefer the laparoscopic method in recent years.

Table II shows the distribution by age. The operation was performed most frequently on patients between 30 and 40 years, but not less than 219 patients were below the age of 30 years.

Table III shows the circumstances under which the operations were done. About half of the operations were done in connection with legal abortions under the same anesthetic while 113 patients underwent post partum sterilization by the laparoscopic technique. More recently, with increasing experience, preferred the latter method also post partum.

The indications for the operation fell mainly into two types. The medical indications for the operation are seen in Table IV. The other 1053 patients all had their applications approved by the Department of Public Health.

As appears from Table V that most of the women had children before they resorted to sterilization. Thirty of the patients had had no pregnancy at all and some of the operations were performed on psychiatric indications.

About 30% of the patients had one or more previous abortions before they were sterilized. This is less than double the rate expected in the population (Table VI).

As seen from Table VII that 20% of the patients had more than one therapeutic abortion before they were sterilized.

As previously shown in Table III about half of the operations are performed at the time of therapeutic abortion. These abortions are not included in Table VII for laparoscopic sterilization the following items of equipment are used: (i) Wolf Operating Laparoscope and Jacobs Palmer with angled optics. (ii) Biopsy for and grasping forceps with blunt jaws and insulated

Table VII Therapeutic abortions prior to sterilization

Therapeutic abortions	1970	1971	1972	1973	1974	Total
One	19	26	31	51	55	182
Two	6	4	6	8	19	43
Three or more	0	1	1	4	1	7
Total	25	31	38	63	75	232

shaft suitable for electrocoagulation. (iii) Verres needle for laparocentesis and insufflation. Long Hegar dilator for manipulation of the uterus. Wolf CO₂-PneuAutomatic chamber for CO₂ insufflation with control of volume and pressure. (iv) Fiberoptic light source and cables.

The anesthetic is chosen with regard to the risk of explosion and all operations were done under intubation anesthesia. The patient is placed in 20–30 degrees Trendelenburg position and 1–3 litres of CO₂ are insufflated to make a pneumoperitoneum. The laparoscope is inserted through an incision in the lower edge of the umbilicus and with the grasping forceps the tubes are lifted away from other structures, especially the intestines. The tubes then are coagulated and divided 1–2 cm from the uterine cornu (Figs 2–3).

Complications

At the very beginning we had some difficulty in making the pneumoperitoneum which is essential for this technique and we had three times to resort to laparotomy due to this technical failure. Before we became familiar with the method we also had some cases with bleeding from vessels in the mesosalpinx. Usually this bleeding can be stopped by electrocoagulation but in 20 cases we had to undertake laparotomy to stop the bleeding which however was not severe in any case. In our series we had three severe complications. A 26-year-old woman came to the hospital 10 days after laparoscopic sterilization with diffuse peritonitis due to a lesion in the rectum. This particular patient was at the time treated with corticosteroids for disseminated lupus erythematosus and we think that either this disease or the treatment might have been contributory to the complication. Two other patients had a lesion of the intestine, one followed by peritonitis. These complications warned us to stay well away from other structures while doing the electrocoagulation.

RESULTS

Despite the above mentioned complications our experiences with the laparoscopic method for tubal sterilization are good. One of the greatest advantages of the operation is the short hospital stay (Table VIII). Most of the patients left the hospital on the first or second postoperative day and when discharge was postponed it usually was due to intervening holidays, the patients' wishes.

Table VI Spontaneous abortions prior to sterilization

Spontaneous abortions	1970	1971	1972	1973	1974	Total
None	22	36	47	55	79	239
1	7	10	13	14	17	61
2 or more	11	7	6	5	13	42
Total	40	53	66	74	109	342

Table VIII Hospital stay after operation

	Days after operation						
	1	2	3	4	5	6	7 (or more)
Laparoscopic sterilization (Palmer)	449	325	165	32	14	5	2
Resectio tubarum A. M. Pomeroy				5	7	1*	13*

Table IX Failures

Year	Name	Pregnant after (months)	At laparoscopic re sterilization found
1971	M S L	14	Right tube not coagulated
1971	G H	12	Right tube not coagulated
1971	K E	21	Right tube not coagulated
1972	L R	18	Right ligamentum rotundum divided
1972	A L E	9	Left tube not divided
1972	K J	3	Right tube not divided
1972	L H	8	Left tube not divided
1972	A M L	12	Left tube appeared normal
1973	B B	18	Right tube not divided
1973	B H	12	Right ligamentum rotundum divided

diseases being treated at the same time. The time in hospital is now seldom more than 48 hours and can still be reduced if necessary. Another great advantage of the operation is the almost complete absence of postoperative trouble often seen after laparotomy. The patients usually feel only a dull ache in the back or on top of the shoulders due to some CO₂ that has not been evacuated. The unsightly scar both outside and inside the abdomen resulting from laparoscopy places diathermy in a favorable light.

In order to assess the efficiency of the method fully our observation time is rather short but our patients have been observed from 6 months to 5 years. So far we have had ten failures probably due to lack of experience (Table IX). In 2 cases the mesosalpinx had been burned on one side only while in 4 other cases one tube had not been completely divided but partly coagulated leaving a narrow communication between the two ends. In 2 cases the round ligament on one side had been burned and in 2 cases the tube appeared normal and no sign could be seen of the sterilization procedure.

Initially we planned to carry out an X-ray control of the patients or at least Levin's test postoperatively but we changed this decision because of the risk of reopening the tubal stump.

CONCLUSION

With the laparoscopic method of tubal sterilization we have a relatively easy and rapid technique. The contra-indications to the operation are few and previously laparotomies where adhesions may be suspected. In some cases we have done laparoscopies in spite of previous scars but never where extensive adhesions have been observed from earlier operations. With increasing experience the operation is safe and secure and for the patient inconvenience is the least possible.

One advantage not mentioned in previous reports on the subject may be the relatively long and atraumatic distal end of the tube. This lateral end with its small fimbriae might be a better tube to implant in the uterus than the usually short end after Pomeroy sterilization and with better hope of successful reversal of the sterilization.

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lowered (2-12) or normal FSH levels (3-4). No relationship between the amount of bleeding during delivery and pituitary function could be found. In the pathogenesis of Sheehan's syndrome the duration and severity of the shock is the most important and none of our patients had been in shock during delivery.

The high LH level was due to HCG as verified in gel filtration study on Sephadex G 75 (Pharmacia Inc. Sweden). The immuno-reactive LH was mostly (93%) eluted between serum albumins (mol weight 60 000) and globulins (mol weight 200 000) indicating a molecular weight between 60 000 and 200 000. The measured LH level represents HCG (mol weight 100 000) which is not removed from the puerperal serum until two weeks post partum (13) rather than LH (mol weight 30 000). The approximate true LH concentrations were 11 ng/ml before and 15 ng/ml after LRH stimulation. These values are lower than our normal level of 20-40 ng/ml in healthy women during follicular phase.

Our results further indicate that in the puerperium the LRH and TRH stimulation tests can be performed together as has been shown in normal subjects before (7).

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AMNIOTIC FLUID LECITHIN CONCENTRATIONS IN PREGNANCIES COMPLICATED BY HYPERTENSIVE DISORDERS AND INTRAUTERINE GROWTH RETARDATION

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Amniotic fluid lecithin has been measured in 92 samples from 75 patients with hypertensive disorders and normotensive pregnancies complicated by intrauterine growth retardation (IUGR). For the group with hypertensive disorders the lecithin concentrations were not significantly different from a reference material at any time in gestation. Pre-eclampsia associated with moderately elevated blood pressure and complicated by IUGR showed significantly higher lecithin concentrations than any other diagnostic subgroup. The lecithin concentrations showed no relationship to maternal urinary estrogen excretion. The value of serial estrogen measurements in predicting IUGR has been emphasized. An evaluation of amniotic fluid lecithin concentration in relation to respiratory function has been made. Respiratory distress syndrome may occur even in growth retarded babies.

INTRODUCTION

Hypertensive disorders in pregnancy with and without intrauterine growth retardation (IUGR) and normotensive IUGR carry a substantial risk of fetal death in utero due to placental insufficiency (8). These pregnancies are frequently terminated prematurely with the added risk of respiratory distress syndrome (RDS) developing in the newborn. There is a clinical impression that IUGR infants rarely develop RDS (27). Other recent reports suggest that intrauterine growth retardation as a result of placental insufficiency may in fact accelerate fetal pulmonary maturation (7, 13, 22). Furthermore maternal hypertension per se may have the same effect (13). In view of the possibility of accelerated pulmonary maturation in such pregnancies it was of interest to investigate the relationship between surfactant levels as measured by amniotic fluid lecithin concentrations, maternal hypertension and the occurrence of IUGR. It was also of interest to study the

relationship between the lecithin concentrations and the maternal urinary estrogen excretion (E_2), which is an accepted method of determining the function of the fetoplacental unit (4, 13, 21, 23).

MATERIAL AND METHODS

Definitions

Hypertensive disorders

A. Pre-eclampsia (48 patients) 1) Blood pressure (BP) at or above 140/90 mm Hg detected during the second half of pregnancy. BP was measured at least twice with a minimum interval of 6 hours. And in addition one of the following criteria: 2) Proteinuria in a morning specimen of 1/4% (Esbach) or more. 3) Generalized edema or weight gain of 2 kg/week or more.

B. Essential hypertension (3 patients) BP at or above 140/90 mm Hg recorded before and during pregnancy.

C. Pregnancy hypertension (16 patients) BP at or above 140/90 mm Hg appearing at any time during pregnancy without other significant signs and symptoms of pre-eclampsia.

In the test B and C are considered together and termed pregnancy hypertension.

The lecithin concentrations were correlated to the degree of maternal hypertension in the following manner:

Moderate hypertension (52 patients) BP \geq 140/90 mm Hg and $<$ 160/110 mm Hg.

Severe hypertension (15 patients) BP \geq 160/110 mm Hg.

Intrauterine growth retardation (25 patients)

Birth weight at or below the 3rd percentile according to the classification of Bjerkedal et al. (3). 23 out of these 25 small for gestational age infants were considered dysmature.

80 samples of amniotic fluid from 67 patients with hypertensive disorders and 12 samples from 8 patients with non-hypertensive IUGR were collected by transabdominal amniocentesis or at the time of Caesarean section. The 80 samples from the group with hypertensive disorders have been compared statistically at weekly inter-

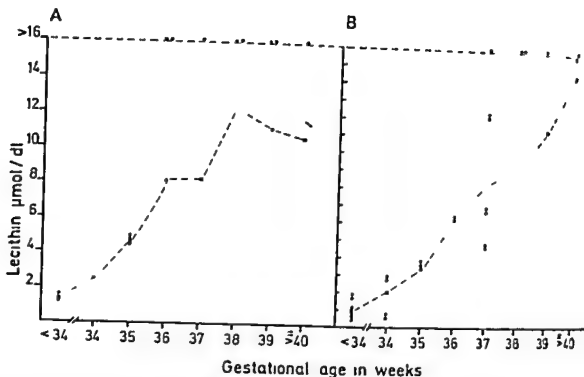


Fig 1 (A) Amniotic fluid lecithin concentration in 80 samples from patients with hypertensive disorders and

(B) in 83 samples from the reference material plotted against gestational age. $\times \times$ Median values for both groups.

vals using the Wilcoxon two-sided two-sample test with a reference material obtained during the last 8 weeks of pregnancy. These samples were from normal pregnancies or from pregnancies where abnormalities were considered insignificant for fetal pulmonary maturation. The same material was used in a previous report (24). Gestational age has in the text been given in completed weeks, i.e. 39 weeks = 273–279 days.

Amniotic fluid lecithin was correlated to the various clinical subgroups and to E_3 . For this purpose the lecithin concentrations were divided into three groups:

Low lecithin (11 patients): Concentrations at or below half of the median value of the reference material.

High lecithin (11 patients): Concentrations twice the median value of the reference material or more.

Appropriate lecithin (53 patients): The remaining samples.

Comparison with the median value of the reference material was made at the corresponding week in gestation for each sample.

$E_3/24$ hours was measured in 70 pregnancies by the method described by Brown et al. (5). Concentrations based upon a maternal of normal pregnancies (4) were classified as follows:

Normal E_3 : Concentrations above -1 standard deviation (SD).

Subnormal E_3 : Concentrations between -1 and -2 SD.

Low E_3 : Fall or not satisfactory rise in concentrations with one or more recordings below -2 SD.

In the majority of patients evaluation of E_3 was based upon more than two measurements. Evaluation of only one measurement was not attempted. Assessment of all

estrogen concentrations was made at the appropriate time in gestation.

Lecithin has been measured quantitatively in centrifuged amniotic fluid as previously reported (18). Unit of substance has been given in micromoles per 100 ml of amniotic fluid ($\mu\text{mol/dl}$). Assessment of respiratory function, the diagnosis of RDS and transitory RDS requiring supplemental oxygen for less than 4 hours has been as previously defined (18).

All cases with uncertain menstrual histories have been excluded. This also applied to cases where an obvious discrepancy existed between the expected date of delivery calculated from the menstrual history and the maturity of the newborn recorded by an independent paediatrician.

RESULTS

The lecithin concentrations for 80 samples from 60 patients with hypertensive disorders are given in Fig. 1A and the results of 83 samples from the reference material are given in Fig. 1B. There is an obvious increase in the median values for both groups with time, but also a very wide range. Statistical analysis of the results at the same ages of gestation for both groups gave no significant differences at any stage.

The correlation between the lecithin concentrations divided into high, appropriate and low

Table I Relationship between the lecithin concentrations classified as high appropriate and low for gestational age, neonatal age, respiratory distress and the various diagnostic groups of hypertensive disorders and intrauterine growth retardation

	No of patients	High lec	Approp lec	Low lec	RDS
Normal without IUGR	35	2	26	7	3
Normal with IUGR	13	6	7	0	0
Pregnancy hypertension without IUGR	15	1	13	1	0
Pregnancy hypertension with IUGR	4	1	1	2	3
Only hypertensive IUGR	8	1	6	1	0
Severe hypertension	15	1	11	3	4
Moderate hypertension	52	9	36	7	2

gestational age and the diagnostic subgroups is summarized in Table I. For preeclampsia with and without IUGR there is a difference in the distribution of the lecithin concentrations. There were 13 infants with IUGR in this category and all had either high or appropriate lecithin concentrations. This difference for preeclampsia is $\chi^2=12.48$ with a probability of significance $P=0.002$. Pregnancy hypertension with and without IUGR as well as only hypertensive IUGR had distributions of lecithin concentrations which showed no clear deviation from normal. However the number of patients was small in two of these groups.

Among those with hypertensive disorders 9 of 10 with high lecithin concentrations were found in pregnancies with moderate hypertension. There were none with severe hypertension among the IUGR infants who had high lecithin concentrations. Six infants developed RDS and four were from pregnancies with severe hypertension. Three of the infants had IUGR. In the 75 pregnancies there were 1 fetal death, 1 intrauterine death, 74 liveborn babies and no neonatal deaths.

The relationship between E_3 in 70 pregnancies and the distribution of lecithin concentrations is given in Table II. Both high and low lecithin concentrations were more frequently seen in the group

with low E_3 than in those with normal or subnormal E_3 . 25 pregnancies with low E_3 resulted in the delivery of 17 IUGR infants (68%). Four of the 6 babies with RDS occurred in the low E_3 category and three of these were from pregnancies with severe hypertension. There were 6 cases of high lecithin in the low E_3 group and none occurred in pregnancies with severe hypertension.

Fifty samples of amniotic fluid were obtained from 50 patients within one week of delivery. These pre-delivery lecithin concentrations together with serial measurements in three pregnancies with IUGR are shown in Fig. 2. Lecithin concentrations $>4.6 \mu\text{mol/dl}$ have previously not been associated with RDS except in maternal diabetes mellitus (24). Six babies of 12 with pre-delivery lecithin concentrations $<4.8 \mu\text{mol/dl}$ developed RDS. In two of these the distress was transitory only and of a more severe kind in the remaining four. There were no signs of RDS in the other 6 infants with lecithin concentrations $<4.8 \mu\text{mol/dl}$ or in the 38 with concentrations $>4.8 \mu\text{mol/dl}$. The same was the case with the 24 infants not included in Fig. 2.

The three IUGR infants with serial lecithin measurements had low concentrations in repeated samples during the last 4 weeks of pregnancy. Delivery was postponed after each measurement until the

Table II Relationship between the maternal estrogen excretion and the lecithin concentrations classified as high appropriate and low for gestational age, intrauterine growth retardation and respiratory distress

	No of patients	High lec	Approp lec	Low lec	IUGR	RDS
Normal E_3	8	2	23	3	2	1
Subnormal E_3	17	1	13	3	6	1
Low E_3	25	6	14	5	17	4

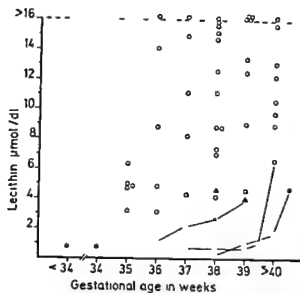


Fig 2 Amniotic fluid lecithin concentrations in 50 samples obtained within one week of delivery related to gestational age x—x Serial lecithin analysis in pregnancies with growth retarded infants O no respiratory distress ● respiratory distress ▲ transitory respiratory distress

risk of RDS developing was considered reasonably small and the distress which occurred in two babies was not of a severe nature. The three very early deliveries were in pregnancies with ominous rise in blood pressure and with fulminant eclampsia in one patient. These three infants all developed RDS. One of them had IUGR.

The data of the calculated lecithin/sphingomyelin are not included since no additional information could be obtained from the material studied in the report.

DISCUSSION

The lecithin concentrations in pregnancies complicated by hypertensive disorders in this study show a wide scatter with some concentrations quite high around 35–36 weeks of gestation and some fairly low close to term. This picture has become familiar and the present material has been shown not to be statistically different from the reference group of similar size. There is therefore no indication that maternal hypertension as such is capable of accelerating fetal pulmonary maturation as measured by amniotic fluid surfactant (12).

There is however widespread clinical agreement that RDS is not a feature of IUGR (7, 22).

Usher (27) even claims that he has yet to see an infant with fetal malnutrition develop respiratory distress syndrome. The association of good lung function in dysmature babies has been linked to the existence of a stressed situation before birth which has been thought capable of an elementarily augmenting lecithin synthesis. In the present material the finding of a highly significant relationship between mature lecithin concentrations in pre-labour patients who gave birth to babies with ILGR is in agreement with this view. Among the 13 infants in this category there were none with low lecithin concentrations for gestational age or respiratory difficulties. For this group therefore sufficient work may have existed to augment lecithin synthesis through endogenous steroid production. A comparison may be made with the results whereby corticosteroid administration to the mother or the fetus seems to accelerate fetal pulmonary maturation (9, 15, 16, 17).

The other infants with IUGR in this study had lecithin concentrations not different from the reference material. No evidence of accelerated pulmonary maturation was therefore found in the groups. Pulmonary maturation was even retarded in some cases. Since small for gestational age infants comprise a heterogeneous group it is possible that the cause of the growth retardation in the pre-labour pregnancies was more clearly established and that the moderate hypertension associated with it was more capable of augmenting lecithin synthesis than either growth retardation associated with severe maternal hypertension or normal blood pressure. Whatever the cause IUGR infants may certainly develop RDS. As shown in Fig. 2 serial amniocentesis was made in three patients who delivered IUGR babies. The fact that several samples in each patient had low lecithin concentrations clearly points out that pulmonary maturation may be delayed in some of these infants. Indeed two of these three plus one more IUGR infant delivered before 34 weeks did develop RDS. There are no other reports of such infants developing RDS (14, 22). It would in fact be surprising if a heterogeneous clinical entity as IUGR invariably was blessed with a favourable outcome in this respect.

Attempts were made to establish a relationship between high and low lecithin concentrations and fetal renal estrogen excretion. As shown in Table II, no clear relationship existed. This is in agreement with the findings of Freeman et al. (11). Both groups

lecithin concentrations were more frequently in the low E_3 group than in those with normal or subnormal E_3 . Of the 6 infants with high lecithin all were growth retarded and 5 were in the group of preeclampsia with moderate hypertension. Of the 5 infants with low lecithin/low E_3 none had the triad of IUGR and maternal preeclampsia and moderate hypertension. This appears to give the impression that such a situation may be sufficient to bear any direct relationship to the amniotic lecithin level and its value was confined to predict fetal growth or the lack of it and together with the lecithin concentrations helped determine the optimal time of delivery. In previous reports (19-25) lecithin concentrations $>4.6 \mu\text{mol/dl}$ with the frequent exception of maternal diabetes have not been associated with RDS. In this series of experiments 50 patients had a spontaneous abortion within one week of delivery. 38 had lecithin concentrations $>4.8 \mu\text{mol/dl}$ with no signs of RDS and 6 babies with RDS all had concentrations $<4.8 \mu\text{mol/dl}$ confirming our previous experience. Amniotic fluid lecithin therefore accurately seems to reflect both the state of maturity of the fetal lungs and the amount of surfactant reserve present. With low reserve stable alveoli are in addition dependant upon undisrupted surfactant synthesis during the final stages of pregnancy and after delivery. The mechanism of surfactant synthesis may be damaged by intrapartum complications (6, 12, 24, 26). The combination of severe maternal hypertension/low E_3 in the presence of low lecithin concentrations was found in three of the 6 cases of RDS studied in this report. It is likely therefore that such complications are capable of producing RDS with greater frequency the lower the amniotic fluid lecithin concentration or surfactant reserve is. This likelihood is however considered small with lecithin concentrations $>4.6 \mu\text{mol/dl}$.

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CERVICAL CANCER IN WOMEN BELONGING TO A CYTOLOGICALLY SCREENED POPULATION

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Abstract. Women belonging to the cytologically screened population of Stockholm in 1968 to 1974 and developing cervical cancer of stage I to IV were studied. The purpose was to find out the number of women in whom the cancer of preclinical stage was not detected at routine screening and the reasons for this fact. It was found that 34 out of 143 women had never been cytologically screened. The remaining 143 women had been checked at mass screening at private specialists or hospitals. In 51 screened women the cancer was not detected until the women themselves attended a doctor because of symptoms. Thus 34 women or 48% of the series the cancer escaped detection at an asymptomatic stage. Errors causing a delay in interruption of the follow up of patients with suspicious smears or colposcopic atypia were observed in six. Sixty-four patients or 45% of all screened women had had at least one negative smear within 4-5 years prior to discovery of the malignancy. Out of these patients had got a negative smear within 3 years. What might be the true evaluation of these negative smears and their influence on the continued management of the women was important. In view of the results of this study the 4 years interval of rescreening practiced in Stockholm seems to be too long. Moreover the statement supported that the value of health screenings is hampered by the fact that the people most at risk are least likely to attend.

INTRODUCTION

The value of population health screenings probably lies in the rate of the total female population in which the least likely to attend. In a Norwegian study the incidence of invasive cervical cancer among never screened women was estimated to be the same as the rate of the total female population in Sweden (11). In Sweden cytological health screening has been practiced since 1958 and from 1968 there has been a mass cytological screening programme going on in Stockholm with the aim of screening the women every 4th year.

The load on the gynecological clinics and cytological laboratories continuously increases because of frequent checks up of a growing number of women with suspicious smears. The risk of failure in the management of these cases might be considerable. So for instance the presence of a high amount of false negative smears has been reported (3, 4, 11).

This study was undertaken in order to find out the frequency of cervical cancer or its preclinical stage escaping detection at routine screening and the reasons for this. The women invited to the cytological population screening of the city of Stockholm and developing cervical cancer of stages I to IV were studied.

Firstly the number of non attendance was estimated. Secondly the frequency and type of factors in the screening routine causing a delay or mistaken diagnosis were analysed. The aim was finally to judge whether a screening interval of 4 years might be reasonable.

MATERIAL AND METHODS

The material consists of those women invited to the cytological mass screening of the city of Stockholm in the years 1968 to 1974 in whom cervical cancer of stages I to IV was detected in this period. During these 7 years a total of about 230 000 women born in 1920 to 1939 and in 1914 to 1915 were invited to the population screening at least once. In 1968 the first year of screening women born in 1930 to 1934 were asked to attend. That year cervical cancer was detected in 6 of these women. From next year on women born in 1935 to 1939 who got cervical cancer were included in the series since they belonged to the population which had been screened. In 1970 patients with cervical cancer born in 1925 to 1929 were added and so on (Table I).

Patients were looked for in the registry of the radiotherapy clinic Radiumhemmet and as a cross check

Table 1 The number of those women belonging to the population of cytological screening in whom cervical cancer was observed and the years of diagnosis

Year of diagnosis	Year of birth						Sum
	1930-34	1935-39	1925-29	1914-15	1900-21	1902-4	
1968	6						6
1969	11	5					16
1970	13	1	11	12			37
1971	5	1	9	11	7		33
1972	5	4	4	5	3	14	35
1973	5	5	7	4	2	8	31
1974	7	5	1		2	4	19
Sum	52	21	32	32	14	26	177

in the registry of cancer at the Swedish Board of Health and Welfare. Information about patients registered in 1968 to 1971 is recorded on a computer system. Since information about patients in the subsequent years has not yet been fed into the computer system, these women were searched for manually at the Registry of Cancer. In this way the case records of 182 patients were collected and studied. The addresses of the patients at the year of invitation were checked either by asking the patients themselves or at the church registries. It was noticed that 5 women moved into the city the same year as they were to be asked to attend the screening. Due to a delay in the information of registration of new inhabitants, these women did not receive an invitation to the mass screening. They were thus excluded. The remaining 177 women were looked for in the registry of the cytological population screening. Information of patients not present in this registry was obtained either from the case stories and/or from the women themselves. Finally private gynecologists contributed with information concerning earlier cytological checks.

Control of the material

When comparing the names of patients of the registry of the Radiumhemmet with those of the computer lists, it was observed that 18 patients (10%) were missing on the

latter. On the other hand, 13 patients with dysplasia cancer in situ were wrongly present on the computer. In the registry of the Radiumhemmet, data on 6 of these concerning either addresses or the year of birth was to be incorrectly recorded and the women were primarily excluded. However, the true conditions were later confirmed on the computer lists.

Table 2 Distribution of microinvasive and invasive cervical cancer

	Stage of cervical cancer		Sum
	IA	IB-IV	
Number of patients attending the mass screening	25	73	98
Number of patients screened elsewhere	8	37	45
Number of patients never cytologically examined	3	31	34
Sum	36	141	177

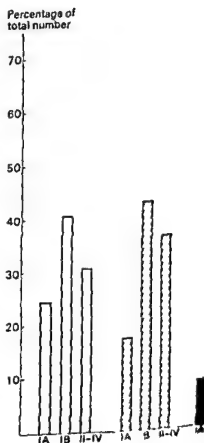
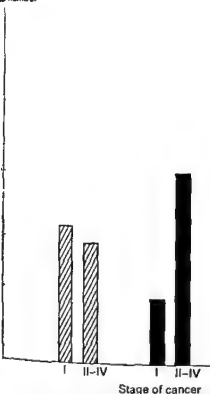


Fig. 1 The percentage of women checked for different stages of cervical cancer.

percentage of
total number

1 The stage of cervical cancer observed when the patient consulted a physician because of symptoms (hatched bars) 2 patients never cytologically checked (solid black bars)

RESULTS

During the years 1968 to 1974 cervical cancer of stages I to IV was detected in 177 women being divided into the cytological population screening of Stockholm (Table I)

Chance

Eighty-eight of these women did attend the screening. Among the rest 45 patients had had one or more smears at a private specialist, at hospitals or elsewhere. In fact 141 women had been cytologically examined at least once within 6 years and two within 8 years prior to the detection of the cancer. The remaining 34 patients had probably not had a smear taken previously. In 4 cases it cannot be determined since information is lacking in the case files and the women are dead (Table II)

Distribution of the cancer related to stage and the way of its detection

Fig. 1 shows the percentage distribution of cervical cancer related to stage at the time of detection of patients attending the mass screening of women checked somewhere else and of women never cytologically examined. There is no distinct difference in distribution of stage of cancer between women attending the mass screening and women checked elsewhere. However the high percentage of cancer of stage II or more in women who had never been screened is noteworthy. This is also prominent in Fig. 2 showing the percentage distribution of cervical cancer by stage in those screened (mass screening and elsewhere) and unscreened women that consulted a doctor because of symptoms. Out of 87 women with atypia in the smears 47 were sent to Radiumhemmet within 1 year's time. In 51 screened women the malignancy was not detected until symptoms made the patients visit a doctor. Thirty-four of these had attended the mass screening (Table III)

Failures

Two patients only neglected a follow-up being recommended because of a suspicious smear. Physicians' mistakes were observed in 23 cases. In 6 of these the follow-up was interrupted since the women got a negative smear after one or more positive ones. In three cases there was an atypical colposcopic appearance but since the smear was negative no check-up was carried through. For unknown reasons there was no check-up in the remaining 14 women in spite of atypia. In 14 patients the malignancy was not detected until symptoms made the women visit a doctor. The remaining women went for another routine check later (Table III)

Negative smears

Smears judged as belonging to Pap II are not referred to as negative in this study. Women with negative smears earlier than 4-5 years before the cancer was detected are not included.

Out of 98 women attending the mass screening 30 had got a negative smear within 4-5 years prior to detection of the cancer and out of 84 women checked either both at mass screening and elsewhere or only somewhere else 39 got a negative smear at the check elsewhere (Table IV). Five of these women had had a negative smear both at the mass screening and at the check elsewhere. On

Table III *The way of detection of the cervical cancer in 143 cytologically screened patients*

Patients with	The cancer was detected			
	During follow up because of suspicious smear			When the patient attended a doctor because of symptoms
	Within 1 year	1-2 years	>2 years	
Suspicious smears	47	8	7	3
Suspicious smears lost by mistake				7
Suspicious smears or colposcopic views and negative smears lost by mistake				4
Negative smears				19
Total	62			30
				31

patient got a negative smear both at the first and second control at mass screening. In one woman only the cancer was discovered at the second control at mass screening about 4 years after the first one.

By adding the women neglecting the mass screening but checked elsewhere to those attending the mass screening it was found that 64 patients or 45% of the screened women had had a negative smear within 4-5 years prior to detection of the malignancy (Table IV). This number includes 7 women with a negative smear at private checks in whom atypia was later observed at the mass screening and 4 patients with a negative smear at the mass screening in whom atypia or cancer was later detected by private specialists. Five women with one or more negative smears after consultation due to cancer *in situ* performed 1 to 7 years earlier later

developed invasive cervical cancer. Table I shows the time interval between the last negative smear and the date of detection of the cancer in a later stage. In 83% of the women the cancer was detected within 3 years.

Later on 23 of the patients with negative smears went to another routine check at which malignancy was observed. The remaining 41 women did not seek medical advice until later and gave rise to symptoms (Table III).

DISCUSSION

Attendance and stage of cancer detected

Forty percent of the women invited to the mass screening of Stockholm failed to appear (11). The same figure was observed in this study. In 45% of the women developing cervical cancer

Table IV *The way of cytological control and the amount of patients with a negative smear within 4-5 years prior to detection of cervical cancer*

Smears taken	Total number of patients	Number of patients with negative smears 4-5 years prior to the diagnosis of cancer		
		At mass screening	Both at mass screening and elsewhere	Elsewhere
At mass screening only	59	14		3
Somewhere else	45			11
At mass screening and somewhere else	39	11	5	3
Percentage of patients with negative smears	64	25.5		41
Number of patients with negative smears	45	31%		

Table V The time interval between the last negative smear and the date of discovery of the cervical cancer related to stage

Stage of cancer	Time in years			
	0-1	2	3	4-5
I	2	3	2	
II	5	12	9	7
III-IV	6	8	6	4
Total	13	23	17	11

attendants of the mass screening. The finding that a good half of these women were regularly checked somewhere else is in accordance with earlier observations (2, 5, 11). However, the proportion of true non-attendants among women of Stockholm getting cervical cancer (19%) is quite high compared to the one observed in total population invited to cytological health screenings (<6%) (8). This result supports the proposal that the women most at risk are the least likely to attend health screenings.

Patients with micro-invasive and occult cervical cancer are included since they were treated at the radiotherapy clinic. The majority of the cancer of stage IA was diagnosed at mass screening and at routine checks elsewhere. It was seen in 3 patients only of those never checked (Table I, Fig. 1). The cancer of the unscreened women generally had progressed to a more serious stage than that of those screened women in whom the cancer was detected when they attended a doctor because of symptoms. This discrepancy also noted by Pedersen in Norway (11) accentuates the seriousness of non appearance and/or indifference of the never screened women.

The significance of dysplasia and cancer in situ as a precursor of invasive cancer is not fully clear and has even been doubted (7). The main questions deal with the length of the preinvasive stage and the proportion going to invasion. A follow up of patients with dysplasia or cancer in situ without any biopsy or treatment is hard to perform for ethical reasons. In the 25 women of this study on whom follow up was incorrectly interrupted such an outcome was realized. However, as long as we do not know the percentage of suspicious smears never

developing into invasive cancer the problem can not be solved.

In two counties of San Diego the cases of invasive cervical cancer diagnosed in 1967 were studied and preventable factors recorded by Martin (9). His purpose was to estimate the efficiency of a 10-year screening project. It was observed that in all but 3 out of 76 cases there were probable errors. Considering the result of the present study that in 36% of cytologically checked women the malignancy was permitted to progress until it gave rise to symptoms, Martin's statement of a low level of efficiency of screening activity might be true.

A reason for the low efficiency of screening activity might be the heavy load of gynecological clinics with a continuous increase of patients repeatedly checked due to suspicious smears. It might happen that the doctor classifies a suspicious smear preceding a normal one as due to coincidence or inflammatory changes and interrupts the follow up. Since 8 women developing invasive cancer were lost in this way it is reasonable to expect a higher number of cancer in situ escaping detection out of similar reasons.

The negative smear

The presence of a rapidly appearing type of cervical cancer according to the theory of Ashley would lower the effectiveness of screening (1). Such a cancer might have been more frequent among the 64 women with previous negative smears compared to the remaining screened women. However, some workers observing patients with negative smears preceding an invasive cervical cancer discussed the possibility of laboratory and sampling failures (2, 3, 6, 9, 11). Whatever might be the correct evaluation of the negative smears of this study a screening interval of 4 years seems to be too long.

Some authors point to the fact that the incidence of dysplasia and cervical cancer progressively decreases during repeated re-screenings instead of remaining constant as expected (4, 13). Coppleson et al. proposed that this is due to the entry of a continuously decreasing number of cases previously falsely recorded as negative. In the present series about half of the smears proved to be incorrectly evaluated (12). Recently a proposal was made to take two smears at a time to diminish the risk of sampling failure and error of evaluation of the material (14). In fact 86% of improvement of

detection of abnormal cytology was reached in this way

In conclusion it can be stated that with a higher efficiency of screening activity the 51 women not detected might have been caught earlier and saved from highly malignant disease (Table III)

ACKNOWLEDGEMENT

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ASSESSMENT OF ANTERIOR PITUITARY FUNCTION DURING THE POST PARTUM PERIOD

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Abstract In order to assess anterior pituitary function during the puerperium 10 women were studied by 14 hr venous LRH and 10 TRH stimulation tests within 10 days post partum. The basal FSH level (150-340 mU/l) was within the normal non pregnant range for the follicular phase of the menstrual cycle (50-350 ng/ml) and did not increase after 100 µg of synthetic LRH. The TSH (4.8 µU/ml) was high and increased after 200 µg of synthetic TRH about twofold. Obstetrical parameters (milk excretion pregnancy complication type of delivery or the amount of bleeding during delivery) were not associated with significant changes in FSH or TSH levels or the responses to TRH stimulation.

Although anterior pituitary function during menstrual cycles has been evaluated repeatedly the puerperal period apart from extensive physiological changes has received little attention. The post partum period is a sensitive one for a woman; for example it has been estimated that 4 per cent of women who lose more than 800 ml of blood during labour suffer some transient ischaemic injury to the anterior pituitary although post partum panhypopituitarism (Sheehan's syndrome) is rare (8). The present study was performed in order to evaluate anterior pituitary responsiveness to luteinizing hormone releasing hormone (LRH) and thyrotrophin releasing hormone (TRH) in puerperal women with special reference to various types of pregnancy and delivery.

MATERIAL AND METHODS

Ten women volunteered for this study. Clinical data are shown in Table I. For 10 of them a separate LRH stimulation test, for 6 cases a separate TRH test and for 4 cases a combined LRH and TRH test were performed within 2-10 days post partum.

The stimulation test was started at 07.30 after an overnight fast. The patients were in supine position during the test and a venous cannula was used. Three basal samples were taken 30 min, 15 min and immediately before the intravenous injection of 100 µg synthetic LRH and/or 200 µg synthetic TRH (Ferring AB Sweden) in 5 ml sterile water. Blood samples were taken 5, 10, 15, 20, 30, 40, 60, 90 and 120 min later. Serum was separated and stored at -18°C until assayed.

Serum follicle stimulating hormone (FSH), luteinizing hormone (LH) and thyrotrophin (TSH) were determined from a sample volume of 100 µl by a double antibody radioimmunoassay.

Purified hormones and antisera were kindly furnished through the courtesy of the NIAMDD. The hormones were labelled with ^{125}I (IMS 30, Amersham) at specific activities ranging from 100 to 700 mCi/mg. The antisera were used in dilutions recommended by NIAMDD. In the semilog plot the inhibition curve was linear between 10 and 500 ng of FSH (LER 907) per tube, 5 and 100 ng of LH (LER 907) per tube and 0.2 and 50 µU of TSH (MRC 63/89) per tube. The immuno-complex was precipitated by sheep antirabbit serum. Normal rabbit serum and 0.01 molar EDTA (final concentration) were added to obtain complete precipitation. After 24 hrs the precipitates were separated by centrifugation. All the determinations were done in duplicate. The intra assay coefficient of variation varied from 7 to 12% and the interassay coefficient of variation from 10 to 15%.

RESULTS

Before the injection of LRH the individual FSH values varied from 155 to 340 ng/ml and after the administration of LRH from 141 to 345 ng/ml. LRH stimulation did not increase the level of FSH in any patient in the series (Table II). The unresponsiveness of the anterior pituitary remained the same if the LRH test was performed alone or combined with the TRH test. Various obstetrical parameters were not associated with significant differences in

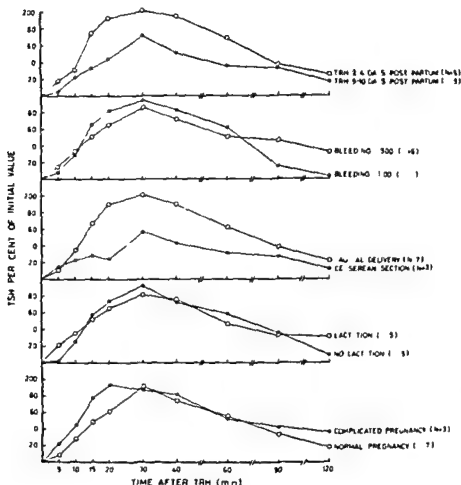
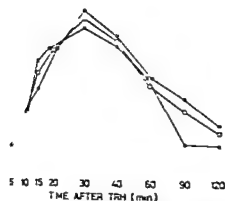


Fig 1 TSH level (2 of initial value) after low dose administration of TRH 2 µg in various groups of patients

Table 1 Volunteers investigated with intravenous LRH and/or TRH stimulation tests during postpartum period

Joun	Age	Parity	Type of pregnancy	Delivery		Bleeding (ml)	Milk excretion	Test	Days postpartum
				Pregnancy week	Type				
1	22	1	Normal	39	Vaginal	<500	+	LRH	3
2	24	1	Normal	39	Vaginal	<500	+	LRH	5
3	27	1	Pre-ecl	38	Vaginal	<500	-	LRH	6
4	35	1	Rh imm	33	Section	1200	-	LRH	5
5	34	1	Normal	39	Section	700	+	LRH	4
6	30	1	Normal	40	Vaginal	1000	-	LRH	4
7	20	1	Normal	41	Vaginal	<500	+	LRH	1
8	25	1	Normal	40	Vaginal	1100	+	LRH	1
9	30	6	Rh imm	37	Section	1700	+	LRH	3
10	24	1	Pre-ecl	37	Vaginal	500	-	TRH	4
11	25	1	Normal	40	Vaginal	1100	+	TRH	1
12	18	1	Normal	40	Section	<500	-	TRH	4
13	19	1	Normal	38	Vaginal	100	-	TRH	8
14	30	6	Rh imm	37	Section	1700	+	TRH	1
15	30	1	Normal	38	Vaginal	1200	-	TRH	10
16	24	1	Pre-ecl	37	Vaginal	<500	-	Ceased	5
17	24	2	Pre-ecl	39	Vaginal	<500	+	Ceased	6
18	26	1	Normal	39	Section	<500	+	Ceased	8
19	24	5	Normal	38	Vaginal	<500	-	Ceased	8
20	30	3	Normal	39	Vaginal	<500	+		



TSH level (% of initial value) after intravenous injection of TRH (200 μ g) alone or together with LRH (100 μ g) ●=all the test (N=10) ○=combined test (N=6) x=separate test (N=6)

al FSH levels although they were somewhat d in patients following caesarean section and in patients with in utero or no milk secretion

obtained "LH" values were high ranging 0 to 1430 ng/ml with no response to LRH stimulation. This was mainly due to human chorionic gonadotrophin (HCG) as verified with radioimmunoassay studies (see discussion)

basal level of TSH varied within 3.3 to 8.8

The absolute TSH values as the means and after TRH stimulation are shown in Table 1

Every volunteer responded to TRH stimulation, the greatest increase in TSH level being 171% and the smallest 148%.

The relative sensitivity of the pituitary to TRH was not related to obstetrical parameters although the response was slightly depressed in patients after caesarean section (Fig. 1).

The pituitary response to TRH was similar if TRH was administered alone or combined with LRH (Fig. 2).

No side-effects were observed after the administration of LRH. After TRH stimulation was complained of by one woman and itching by two women.

After combined LRH-TRH tests no cumulative harmful side-effects were observed. TRH did not improve the milk secretion in any woman.

DISCUSSION

In every puerperal woman the pituitary gonadotrophin (FSH) could be detected and the range of 155-340 ng/ml fell in our normal non-pregnant level during the follicular phase of the menstrual cycle of 50-350 ng/ml. This finding is in accordance with previous results (4) but contrasts with low or undetectable FSH levels in the puerperium reported by some investigators (2, 11). In our volunteers LRH did not stimulate the FSH secretion 2-10 days post partum. Previously Canales et al (1) observed the same pituitary unresponsiveness to intravenous administration of 50 μ g of synthetic LRH within the first 48 hrs post partum but some response 15 days after delivery. LeMaire et al (10) could show some response in LH level after a subcutaneous injection of 100 μ g of LRH in 3 puerperal women 6 weeks post partum but no response 3 weeks after delivery. It remains to be elucidated how long the pituitary tolerance to LRH lasts in puerperal women and what is its cause.

The TSH level during post partum time was high as found by the others (6, 11). The pituitary reaction to TRH in puerperium is maintained. Kannan et al (9) also found about twofold increase in TSH level after 250 μ g of synthetic TRH in 3 subjects tested one week post partum. Somewhat greater responses to TRH have been reported in 3 post partum women studied by Gautvik et al (5).

No obstetrical parameter studied in our investigation was shown to have any influence on the FSH or TSH level or the responses to TRH stimulation. Lactation has been observed to be associated with

Mean FSH (LER 907) and TSH (MRC 63/89) levels before and after LRH or TRH stimulation

	-30	-15	0	5	10	15	20	30	40	60	90	120 min
FSH (ng/ml)	17	217	200	193	214	212	206	206	224	116	208	200
	46	40	43	30	54	42	34	42	58	49	41	42
TSH (%)	53	54	50	59	69	82	89	99	92	81	71	64
	11	16	11	15	16	23	31	24	23	21	20	18

Table 1 The incidence of *Chlamydiae*, *Mycoplasmas* and bacteria in the cervix and the Fallopian tubes (n=21)

	Cervix	Tubae
<i>Chlamydiae</i>	6	1
<i>Mycoplasma hominis</i>	9	0
<i>T. mycoplasma</i>	14	1
<i>Neisseria gonorrhoeae</i>	7	1
Other bacterial pathogens	17	2

the Fallopian tubes as well. One patient with minor symptoms for two weeks before the acute incident had high unchanged CF titres (1/128–1/256) although no organism could be isolated. In these five patients no other pathogenic microorganism was isolated from the tubal exudate.

DISCUSSION

Previous investigators using a laparoscopic technique for the collection of specimens (5) have reported acute salpingitis associated with *Mycoplasma hominis*, *T. mycoplasma*, *Neisseria gonorrhoeae* and some other bacterial pathogens. Our study is too small to allow any comments on a quantitative basis, but the findings are in accordance with earlier reports. Some studies claim that *Chlamydia* only gives rise to silent infection in the lower genital tract and only with minor symptoms (3, 8). Hilton et al. (3) mention a case with prolonged pelvic infection where *Chlamydia* was isolated from the cervix. In epithelial infections, urethritis and cervicitis, the CF test is relatively insensitive as a diagnostic tool. A significant increase in CF antibody titres or an unchanged high titre is therefore suggestive of a deep-seated infection with a massive immunogenic stimulation by the causative agent. The finding of *Chlamydia* in cervical secretions in 6 out of 21 pa-

tients is in accordance with earlier serological evidence of continuing *Chlamydia* infection in five patients with a tubal infection together with the occurrence of *Chlamydia* in the Fallopian tubes in one of the five may imply that *Chlamydia* subgroup A is a causative agent in salpingitis.

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LETTER TO THE EDITOR

I have read the paper entitled "Prevention of imminent premature labour" by O. M. Gummerus and S. Saarikoski, *Acta Obstet Gynecol Scand* 54: 95, 1975, and hope you will publish some comments. The trial is labelled a controlled trial but it does not fulfill the criteria for such a trial. The four modalities of treatment were carried out in four different departments in two separate hospitals and the observations were made by four different staffs on one patient each. It is stated that every other patient was sent to Dept. 1 and every other to Dept. 2. I seriously doubt that the decision to send the patients was made on the basis of criteria for admission to the study had been established.

The same objection applies to the trial conducted at the School Hospital. The treatment was started immediately on admission and there was no attempt made to determine whether the patients were in true progressive labour or false labour. The excellent results obtained with the placebo indicate that a very high percentage of patients must have been in false labour if not we would have used dextrose/water and forget about drugs.

The infusion rate of alcohol is obviously much too low. The infusion rate must have been ca 9 ml of 10% solution per minute and not 0.8 ml. If this is correct, the dosage is similar to the one used by Fuchs et al. (1967) but in our material the treatment was often continued for more than 6 hours after initial arrest of labour contractions recurred. The use of ethanol to prevent premature birth is well known but it is true that there is a paucity of data. However, they are not all as negative as quoted by the authors. On the contrary, there was a controlled study as early as 1935 with very good results (3). I would hope that more carefully designed studies than this one could be carried out in the two institutions before the conclusion is reached that ethanol is useless in threat-

ened premature labour. In particular, I wish the authors would address themselves to the difficult question of distinguishing false labour from progressive labour, which is crucial for studies such as these.

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From Fuchs

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Rejoinder

We thank the editor of *Acta Obstetrica et Gynecologica Scandinavica* for the possibility of letting us make our remarks immediately upon the comments made by Dr. Fuchs on our article. The kind of research presented in our article is difficult because it often takes a long time to get enough material. Therefore, the keeping in hands of the research also becomes difficult. We had the possibility of gathering material from two different maternity hospitals which are the same size and managed along the same principles. One member of the research group was working at the Women's Clinic in Helsinki and one at the Midwifery School Hospital. In this manner, the length of the study could be limited to one year.

The selection of patients for the groups presented in the investigation was performed at random. Each patient herself chose the hospital where she wanted to give birth. The doctor working in the outpatients department of the hospital, who did not

belong to the research group decided after having examined the patient whether she needed hospital care or not. After this the nurse sent every first one of the patients to Department I and every second one to Department II.

False labour cases are expected to exist in our material. But we could not begin to choose our material by eliminating false labour cases after our deliberation. We considered that if the groups were large enough an equal amount of false labour cases would exist in each group thus being of no significance when the groups were compared with one another.

Dr Fuchs quite rightly takes an interest in our maybe a little unclear explanation of what exactly was the infusion rate in our research, yet after a careful reading of the article it becomes clear it was 0.8 ml/min ethyl alcohol + 8.3 ml/min mixture solution. Thus the infusion rate was the same as in Dr Fuchs' own experiments.

When referring to earlier research we have listed only verified experiments. This is why the publications mentioned by Dr Fuchs have not been cited.

We have not stated in our article that the alcohol would be useless in threatened premature labour as Dr Fuchs claims. We only said: On the basis of

the present study it is clear that alcohol even if administered intravenously in fairly large doses not result in better therapeutic results than placebo.

We think that the confidence in alcohol as a potent inhibiting premature uterine contractions has begun to vacillate among obstetricians. We saw point at e.g. the Gallup inquiry on the treatment and therapy of premature labour (1). Eight out of 10 American obstetricians expressed their opinion on the questions presented. Five of them recommended starting the therapy with β sympathomimetics and only two the starting of it with alcohol. One of the latter kept β sympathomimetics as a second line drug.

Clinical practice speaks in favour of our findings. Thank you, Dr Fuchs, for the comments.

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Finland

ANNOUNCEMENTS

International Symposium has been organized on ovulation. The symposium will be held at C S Human Growth and Development Wayne University School of Medicine Detroit on April 1977 (following World Congress Fertul Steril^h Fla.) Forty three national and international will discuss oogenesis folliculogenesis structure and scanning electron microscopy of ovula endocrine neuroendocrine and neuromuscular of ovulation intrafollicular endocrine pro- types of anovulation physical biophysical and en techniques to predict and detect ovulation

ovarian ultrasonics induction of ovulation by clomiphene gonadotropins or GnRH regularizaton of ovulation treatment of cycles with corpus luteum defects or polycys tic ovaries and cervical mucus sympto-thermal methods and lactational amenorrhea in relation to natural family planning A M A accreditation Deadline to submit abstracts of contributed research papers is November 1 1976 For abstract forms and additional information Dr E S E Hafez Reproduction Physiology Laboratories C S Mott Center for Human Growth and Development Wayne State University School of Medicine Detroit Michigan 48701 (Tel 313/577 1011)

CERULOPLASMIN AND COPPER LEVEL IN MATERNAL AND CORD BLOOD AND IN THE PLACENTA IN NORMAL PREGNANCY AND IN PRE ECLAMPSIA

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Copper and ceruloplasmin were assayed in maternal cord blood sera and in the placenta of 10 women in normal pregnancies, in 10 patients with mild and 10 with pre-eclampsia. Copper and ceruloplasmin levels were significantly elevated in the maternal blood of pre-eclamptic patients as compared with normal pregnant women. The placental and cord blood concentrations of copper and ceruloplasmin showed no significant difference, which indicates that their increase in the maternal blood is not of placental origin.

Copper is distributed approximately equally between erythrocytes and plasma, except in pregnancy when the concentration rises in the plasma. 96% of the plasma Cu is firmly bound to an α_2 -globulin as ceruloplasmin containing eight atoms of copper in each molecule (11); a variable but small amount is less firmly bound to albumin and a small quantity is dialysable. An increased Cu level in the serum during pregnancy has been reported by several authors (3, 12, 5). Freedman et al. studied the serum Cu level in healthy women at various months of pregnancy and in pregnant women with various pathological conditions. They found that a progressive increase in the serum Cu level occurred during pregnancy followed by a decrease with return to normal levels only a few weeks after pregnancy. O'Reilly & Loncin (7) found a progressive increase in ceruloplasmin starting from the second trimester of pregnancy and reaching a maximum level at the time of delivery, followed by a drop 6 weeks postpartum. They also reported that blood from the neonate has a mean ceruloplasmin level below the lower

limits of the normal range (4, 6). Zarafonetis & Kales (15) reported that ceruloplasmin degraded serotonin at pH 7.4. O'Reilly (7) suggested that the increasing serum ceruloplasmin during pregnancy could play a role in ensuring that the increasing blood serotonin levels did not lead to increased levels of free plasma serotonin. Despite the increased blood serotonin, there was not a proportionate increase in 5-hydroxyindole acetic acid (5-HIAA) output in the urine because of the degradation of the 5-hydroxyindole, one of the metabolites of serotonin, by ceruloplasmin.

Ahlmark (1) and Swanberg (13) suggested that ceruloplasmin and histaminase serum levels may constitute a very sensitive indicator for the normal course of pregnancy and placental function.

The purpose of this study was to investigate the level of copper and ceruloplasmin in maternal serum, in the placental extract, and in cord blood, and to study the significance of any variation.

MATERIAL AND METHODS

Thirty pregnant women were selected from the maternity department of Ain Shams University hospitals, so that 10 cases represented normal uncomplicated pregnancy as a control series, 10 cases were of mild pre-eclampsia, and 10 cases were of severe pre-eclampsia. This classification of pre-eclampsia was according to that previously described by Ramadan, Sammour, Khalil & Eissa (8).

Maternal blood samples were collected before the onset of labour, whereas cord blood and placenta were taken immediately after delivery. Delivery was in all cases per

and in the placental extract were significantly raised in mild and severe pre eclamptic patients as compared with normal pregnant women. The rise from mild to severe pre eclamptic patients was not significant in the placental extract. Cord blood ceruloplasmin was appreciably higher than the levels of the maternal blood and raised non significantly from normal to pre eclamptic cases (Table II).

Statistical analysis of all the data was carried using Student's *t* test and the equivalent *t* tests were calculated to indicate the significance.

DISCUSSION

Work conducted by Zarafonetes (15) and by O'Leary (7) in connection with the role of ceruloplasmin in oxidation of serotonin (5 HT) and its metabolic product 5 hydroxy indole acetic acid (5HIAA) prompted us to study its concentration in placenta and cord blood in relation to the maternal blood level. This investigation is a continuation of our previous work on serotonin in the maternal sera and the placenta (8). At delivery the copper level in the maternal blood increased significantly in severe pre eclamptic cases as compared with normal pregnant women but in mild pre eclampsia the increase was not significant. These findings agree with those of O'Leary (3), O'Leary et al. (5) and Freedman et al. (6). The increase in copper in maternal serum was reflected either in the placental tissue or in the cord blood. This indicates that the placenta is not the source of the increase in the maternal blood. It may be attributed to its release from maternal tissues such as the liver. The low level of copper in placental tissue also affected its concentration in cord blood which showed a further decrease according to the severity of the toxemia. The concentration of ceruloplasmin was parallel to that of copper where it showed a significant increase in maternal serum, a slight increase in the placenta and a progressive decrease in cord blood in pre eclampsia as compared with normal pregnant women (Table II). Cord blood ceruloplasmin concentration in severe pre eclampsia was more than double the level in normal pregnant women at delivery and also significantly higher than in cases of mild pre eclampsia. The marked change in ceruloplasmin level in ma-

ternal blood with no significant change in placental and cord blood indicate that this rise is not of placental origin as mentioned with respect to copper concentration.

The decreased activity of ceruloplasmin in cord blood corresponds to its copper content and confirms the results of O'Reilly (6) and Markowitz et al. (4).

The high concentration of ceruloplasmin in maternal sera of normal pregnancy and pre eclampsia at delivery should reduce the free unprotected serotonin in these sera yet it was found to be significantly high (8). This can be explained by considering that ceruloplasmin is not the main oxidase of serotonin and its metabolic products. Furthermore monoamine oxidase and its rise in pregnancy did not prevent the increased blood serotonin level from exerting its effects on the uterus and placenta. The significance of the rise of ceruloplasmin in sera of pregnancy and toxemic patients still needs further investigation. The increase may be proportional to the serotonin rise or there may be inhibiting factors in pregnancy toxemia preventing its function.

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Table I Copper concentration in ten normal pregnancies and twenty pre eclamptic patients versus $\mu\text{g}/100\text{ ml}$ serum and $\mu\text{g}/100\text{ mg}$ fresh placenta

	Maternal blood			Cord blood			Placenta		
	Pre eclampsia			Pre-eclampsia			Pre-eclampsia		
	Normal	Mild	Severe	Normal	Mild	Severe	Normal	Mild	Severe
No. of cases	10	10	10	10	10	10	10	10	10
Range	170-200	175-205	195-250	95-115	95-120	90-115	8-17	10-1	1
Mean	183.9	192	227.7	108.6	108.3	103.8	10.1	11.0	11
S.D. (\pm)	11.01	9.81	18.02	6.58	8.66	7.94	1.44	0.81	1
Test of significance (P)	N.S.			N.S.			N.S.		
	$P < 0.001$			N.S.			$P < 0.05$		
	< 0.001			N.S.			N.S.		

vagina with no difficult interference. Anaesthesia when needed was by either local infiltration or pudendal block.

The blood samples were left to clot and serum was then collected by centrifugation and kept at 4°C for enzyme assay within 24 hours. Placental samples were obtained excised and washed according to Ramadan et al. (8) homogenized in an equal volume of ice-cold water then centrifuged and the clear supernatant homogenate was adjusted to a known volume for estimation of both copper and ceruloplasmin by taking 3 ml of the homogenate for copper assay and 0.1 ml for ceruloplasmin assay. The same amounts were taken from the serum for both copper and ceruloplasmin assay. For every sample the assays were carried in duplicate according to the methods of Ventura & King (14) and Ravin (10) for determination of copper and ceruloplasmin respectively in serum and placenta. The results for copper are expressed as $\mu\text{g}/100\text{ ml}$ serum and $\mu\text{g}/100\text{ mg}$ fresh placental tissue. But for ceruloplasmin the results are given as $\text{mg}/100\text{ ml}$ serum and $\text{mg}/100\text{ mg}$ fresh placental tissue.

RESULTS

(1) The concentration of copper in our study showed a non-significant increase when compared with normal pregnancy with mild pre eclampsia but increase in copper concentration was significant in severe pre eclampsia when compared with normal pregnant women and mild pre eclampsia cases.

(2) The cord blood copper concentration was much lower than its corresponding level in maternal serum and it showed a non-significant increase in mild and severe pre eclamptic cases.

(3) The placental copper was remarkably increased in mild and severe pre eclamptic patients when compared with the normal group (Table I).

(4) Ceruloplasmin concentrations in our study

Table II Ceruloplasmin level in 10 normal pregnancies and twenty pre eclamptic patients (results expressed in $\text{mg}/100\text{ ml}$ serum and $\text{mg}/100\text{ mg}$ fresh placental tissue)

	Maternal blood			Cord blood			Placenta		
	Pre-eclampsia			Pre eclampsia			Pre-eclampsia		
	Normal	Mild	Severe	Normal	Mild	Severe	Normal	Mild	Severe
No. of cases	10	10	10	10	10	10	10	10	10
Range	21-43	49-65	62-100	10-19	10-16	10-16	13-18	16-19	1-10
Mean	32.5	57.9	81.2	13.1	12.4	11.9	14.8	17.1	11
S.D. (\pm)	6.95	5.85	7.07	7.68	2.0	1	1.61	1.04	1
Test of significance (P)	$P < 0.001$			N.S.			$P < 0.01$		
	$P < 0.001$			N.S.			$P < 0.01$		
	$P < 0.001$			N.S.			N.S.		

HISTAMINE METABOLISM AND FEMALE SEX HORMONES IN WOMEN

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Oral combined contraceptives did not seem to influence histamine metabolism in females. During treatment with estrogen and progestin hormones in four amenorrhoeic patients there was a tendency towards increasing excretion of histamine (MeHi) followed by a sudden decrease corresponding to changes in the urinary estrogen. The excretion of methylimidazoleacetic acid (MeImAA) was not affected by that of MeHi. The findings support the view that an endogenous surge of estrogen may influence histamine turnover in women. Women of postmenopausal age have about the same histamine metabolism as younger menstruating women. Estrogen medication does not influence the histamine turnover in women with symptoms of hot flushes or sweats but did not influence the histamine turnover.

INTRODUCTION

Histamine metabolism seems to be influenced by the normal menstrual cycle (11). The women in this study showed individual variations in the excretion of both histamine and its metabolites. There was a significant difference in the excretion during the follicular and post ovulatory phase. However the excretion of methylimidazoleacetic acid (MeImAA) showed a tendency to increase at the time of ovulation. Also there seemed to be a correlation between the excretion of estrogen and that of methylhistamine (MeHi). Green et al (7) reported that estrogen might influence the urinary excretion of histamine and MeHi. The cases, however, were few and the analyses were not performed under standardized dietary conditions. The present investigation represents an extension of previous studies on the influence of female sex hormones on histamine metabolism and on the effect of oral contraceptives on ovulation

stimulation with gonadotrophic hormones and estrogen substitution at postmenopausal age.

MATERIAL AND METHODS

Sixteen healthy women were treated with different oral contraceptives over 17 tablet periods and a total of 77 urine samples were collected. In 10 of these women urine was also collected during their normal menstrual cycle (39 samples). The excretion of histamine (Hi) and methylhistamine (MeHi) was measured.

A second group comprised four women with secondary amenorrhoea. Two of the subjects were studied during one period of stimulation with gonadotrophic hormones, one was studied during two periods and the fourth during four periods of stimulation. In this group the excretion of histamine and metabolites was studied as well as that of total estrogen and luteinizing hormone (LH). The progesterone level in plasma was measured to decide whether ovulation had taken place.

A third group included 10 postmenopausal women. Ten of them had subjective symptoms such as hot flushes and/or sweats. The other ten had no subjective symptoms at all (Table I).

In this group of women urine collection was performed twice on two consecutive days and this was repeated approximately four weeks later. Histamine and MeHi in the urine were determined. At one of these days serum was taken for analysis of LH and follicle stimulating hormone (FSH) and estrogen excretion was determined in the urine. The 10 women with symptoms were studied in the same way before and during treatment with estrogen (ethinylestradiol 20 µg per 24 hours) four weeks later.

Urine was collected in 24-hour portions. The urinary samples were immediately placed in a refrigerator at +4°C. When samples for hormone analyses had been withdrawn the urine was mixed with hydrochloric acid (1 M). All collections of urine were performed under standardized dietary conditions (4).

Histamine (Hi) in the urine was measured by a bioassay technique according to Wetterqvist & White (19) and the values given are expressed as µg histamine base per 24

Table 1 *Relevant data on 20 postmenopausal women in group 4*

A-K had no symptoms and treatment L-U had symptoms of hot flushes and/or sweats and were treated as follows:
 - Hot flushes or sweats ++ both symptoms present (+) almost free from flushes and/or sweats 0 no symptoms

Subject	Age (y)	Parity	Weight (kg)	Smoking, cigarettes/day	Time since menopause (y)	Symptoms	Symptoms after treatment
A	50	2	60	<10	3	0	
B	51	3	59	0	3	0	
C	51	2	64	0	3	0	
D	56	3	66	0	2	0	
E	57	1	70	<10	2	0	
F	58	3	65	0	1	0	
G	58	3	64	0	6	0	
H	60	2	64	<10	17	0	
I	60	3	61	0	16	0	
K	65	2	65	0	10		
L	48	2	60	<10	2	++	(+)
M	51	1	62	0	1	+	(+)
N	52	2	59	<10	3	++	(+)
O	54	0	66	0	3	++	(+)
P	55	0	65	0	1	++	(+)
Q	56	0	60	0	3	+	0
R	57	3	61	0	5	++	(+)
S	57	1	60	0	1	+	0
T	58	0	64	0	3	++	(+)
U	58	2	64	0	4	+	0

hours corrected for recovery. The identity of histamine was checked according to Reuse (15).

Methylhistamine (MeHi) in the urine was determined as described by Fram & Green (3), White (20) and Granerus et al. (6). The values are given as μg methylhistamine base per 24 hours corrected for recovery.

1-methyl-5-imidazoleacetic acid (MeImAA) in the urine was determined as described by Granerus & Magnusson (5) with slight modifications (4, 11) corrected for recovery and expressed as mg per 24 hours.

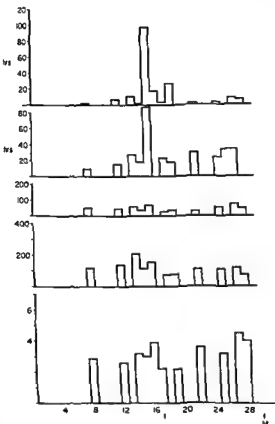
Excretion of luteinizing hormone (LH) in urine was measured according to a technique described by Wide & Porath (22), Wide (21) and Wide et al. (3). The values are expressed in IU per 24 hours. LH in serum was determined according to the same technique and expressed in ng per ml.

The follicle stimulating hormone (FSH) in urine was determined according to Wide et al. (23) and expressed in ng per ml.

The total estradiol excretion was determined as

Table 2 *Urinary excretion of histamine (Hi) and methylhistamine (MeHi) in μg base per 24 hours and in 100 cycles from six women taking oral combined contraceptives (group 1)*

Subject	Compound	Samples	Mean \pm S.E.	Range	Tablet taken
A	Hi	5	46 ± 1	42-50	Fovaryl
	MeHi	5	746 ± 79	167-923	
B	Hi	5	14 ± 2	10-20	Contracept
	MeHi	5	233 ± 25	158-295	
C	Hi	5	16 ± 1	12-19	Contracept
	MeHi	5	730 ± 35	180-934	
C	Hi	5	15 ± 1	12-20	Contracept
	MeHi	5	20 ± 31	151-207	
D	Hi	1	23 ± 7	10-38	Fovaryl
	MeHi	1	711 ± 17	18-146	
E	Hi	5	16 ± 1	10-21	Novum 24
	MeHi	5	707 ± 35	119-901	
F	Hi	5	16 ± 8	1-65	Fovaryl
	MeHi	5	166 ± 5	110-311	



1 Urinary excretion of histamine (H₁), methylhistamine (MeHi), methylimidazoleacetic acid (MeImAA), estrone (Estr) and luteinizing hormone (LH) in a woman

ing to the method of Brown (1) and Littrich (8) and values are given in μg per 24 hours

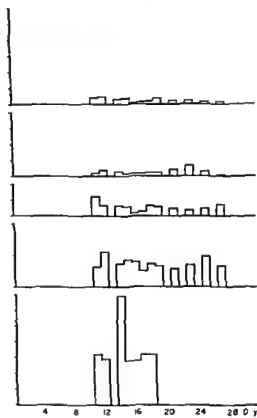
analyses in plasma were performed as described by Johansson (10) modified by Ellingboe et al. (2). The analyses of estrogens and progesterone were as at the Department of Clinical Chemistry, Sahlgren's Hospital.

The first group of women: Conclunett = norethisterone 1 mg + mestranol 0.1 mg; Follinyl = norgestrel 0.5 mg + ethinylestradiol 0.05 mg; Lyndiol mite = lynestrenol 2.5 mg + mestranol 0.075 mg; Anovlar mite = norethisterone 1 mg + ethinylestradiol 0.05 mg; Piloval = quingestrol 0.5 mg + ethinylestradiol 0.05 mg. The second group of women: Humegon = human chorionic gonadotrophin (HCG); Pregnyl = human chorionic gonadotrophin (HCG).

In the second group of women: Linoral = ethinylestradiol 0.05 mg.

Statistical methods

Conventional statistical methods were used for the calculation of means and standard error of means (mean



(age 26, one pregnancy, non-smoking) during one menstrual cycle before (left) and (right) three months later during a period on oral contraceptives (Follinyl).

\pm S.E.). Linear regressions were calculated according to the method of least squares.

RESULTS AND COMMENTS

Six women on oral contraceptives excreted histamine and methylhistamine in the urine (Table II) in quantities within the previously published limits of the normal menstrual cycle (4, 11).

In nine other women where the subjects served as their own controls there were no differences in the mean urinary excretion of histamine and MeHi before and during treatment with oral contraceptives (H₁ before treatment $37 \pm 5 \mu\text{g}/24$ hours; during treatment $32 \pm 4 \mu\text{g}/24$ hours; MeHi before $192 \pm 15 \mu\text{g}/24$ hours; during treatment $195 \pm 13 \mu\text{g}/24$ hours). Fig. 1 illustrates the excretion during one menstrual cycle and a period with oral contraceptives on one healthy female (the sixteenth). No significant changes in urinary histamine or MeHi were observed. At one single day (the 16th)

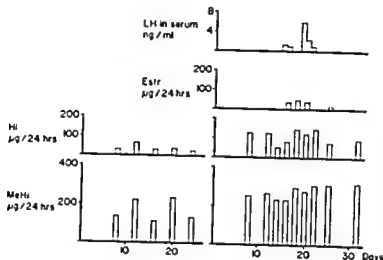


Fig. 2 Urinary excretion of histamine and methylhistamine (MeHi) in a woman (subject F in Table II) while on a period on oral contraceptives (Lynette) (left). Twelve months later the menstrual cycle was examined when she had been without medication for 6 months (right). During this cycle estrogen excretion (Estr) in the urine and luteinizing hormone (LH) in serum were also determined.

excretion of MeImAA rose substantially. This was preceded by a peak in histamine on the eleventh day and peak in MeHi on the twelfth day (right).

Fig. 2 shows on the other hand one woman (subject F in Table II) first studied when taking oral contraceptives (Lynette). She then stopped the medication and the investigation was resumed 12 months later. In this particular case there was a higher urinary excretion of both histamine and MeHi during the menstrual cycle.

Figs 3-6 show the cases in group 2. Four women with secondary amenorrhoea were treated with human menopausal and human chorionic gonadotrophin during altogether eight periods. The doses injected are presented together with the excretion values of hormones and histamine and metabolites in the figures. The excretion levels of plasma progesterone verified ovulation on all occasions.

Three of the patients seemed to react in the same

manner. They showed a tendency to increase urinary output of MeHi in parallel with increased estrogen excretion (Figs 3 and 4). After the maximal estrogen response had been reached there was a decrease in the MeHi values. As shown in Fig. 3 subject 1 excreted increasing amounts of both LH and MeImAA in parallel with an increased LH and estrogen excretion. A subsequent decrease was observed immediately after the period of stimulation. However, in a second course of gonadotropin stimulation of subject 3 no significant increase in MeHi output in connection with the estrogen response could be observed (Fig. 4 right and Fig. 5).

The fourth patient (Fig. 6) reacted with increased urinary excretion of histamine but not of MeHi or MeImAA. In a second attempt to stimulate ovulation there was a weaker hormonal response and a slight if any increase in urinary histamine.

Fig. 7 and Table III show the results from the

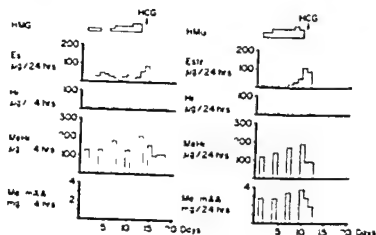
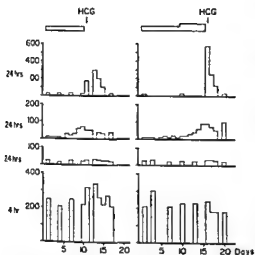


Fig. 3 Urinary excretion of histamine and methylhistamine (MeHi) in a woman (subject 1 in Table I) treated with human chorionic gonadotrophin (HCG) during two courses of treatment (left) and after 12 months (right). Subject 1 was treated with human chorionic gonadotrophin (HCG) 1000 IU of which 500 IU daily was stimulated at the top of the cycle. Subject 2 was treated with doses of 1000 IU of HCG daily. At the peak of the cycle the urinary excretion of LH and estradiol were determined (9000 IU of human chorionic gonadotrophin (HCG)).



4 Urinary excretion of histamine (Hi) methylhistamine (MeHi) estrogen (Estr) and luteinizing hormone (LH) in subject 3 with secondary amenorrhoea (group 2) treated during the first period (left) with 150 I U human menopausal gonadotrophin (HMG) daily and during the second period (right) with doses of 150 or 725 I U daily as indicated at the top of the figures. At the points marked by the arrows she was injected with 9000 I U of human chorionic gonadotrophin (HCG)

nts in the 70 postmenopausal women in group 3 mean excretion values for histamine and MeHi in the group were within the normal limits previously published for fertile women (4, 11) and no differences were found between the subjects with symptoms and those without symptoms. The 10 women with symptoms were then given estrogen orally each day for at least four weeks while the other 10 received no treatment. There was a slight positive correlation between the excretion values for estrogen and urinary histamine in the symptom free untreated group ($r=0.58$) (Fig 7 left). Estrogen treatment did not alter the urinary excretion of histamine and MeHi. Of the hormones analysed only FSH showed a decrease and this observation together with the clinical improvement indicated that the dose given was adequate.

DISCUSSION

The current study illustrates the difficulties involved in trying to understand the role of female sex hormones in histamine metabolism in man. Firstly, it is obvious that there are large individual variations in excretion values of histamine and MeHi in women without abnormal changes in the

internal secretion of sex hormones. It is known that the methodological error is on average $\pm 10\%$ for the analyses of histamine and MeHi (Wetterqvist unpublished).

Secondly, there is an upper limit for the ability of a subject to cope with an investigation of this type. More samples from more women would have been desirable but this was not possible for practical reasons.

Thirdly, the processes underlying the turnover of female sex hormones in the body are not yet fully understood. We have not included studies of releasing factors in our schedule nor did we analyse adrenal secretions. The possibility also remains that the histamine turnover may affect the secretion of female sex hormones via a local or general feedback mechanism.

Fourthly, the principles for the regulation of the total histamine turnover in man are largely unknown. The importance of the composition of the food ingested for histamine metabolism is undisputable (4) but the functional state of the gastrointestinal tract (17) as well as physiological changes in the kidneys may also be of some importance.

Green et al. (7) found increased values for histamine and/or MeHi in the urine of two amenorrhoeic patients in whom an increase in the endogenous estrogen levels had been induced by administration

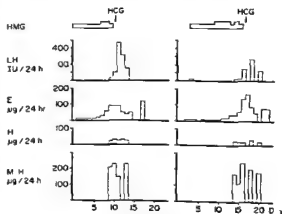


Fig 5 Urinary excretion of histamine (Hi) methylhistamine (MeHi) estrogen (Estr) and luteinizing hormone (LH) in subject 3 with secondary amenorrhoea (group 2) during two further periods of hormone stimulation. During these periods she was treated with 150 or 725 I U of human menopausal gonadotrophin (HMG) daily as indicated at the top of the figures. At the points marked by the arrows she was injected with 9000 I U of human chorionic gonadotrophin (HCG).

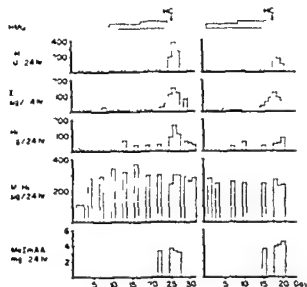


Fig 6 Urinary excretion of histamine (Hi), methylhistamine (MeHi), estrogen (Estr) and luteinizing hormone (LH) in subject 4 with secondary amenorrhoea (group 2) during two periods of hormone stimulation. She was treated with 75, 150 or 225 IU of human menopausal gonadotrophin (HMG) daily as indicated at the top of the figures. At the points marked by the arrows she was injected with 9000 IU of human chorionic gonadotrophin (HCG).

of gonadotrophic hormones. The changes in MeHi values were not impressive whereas the histamine tended to increase. However, there did not seem to be any absolute correlation between high estrogen values and a high urinary histamine output, nor did Green et al. collect urine under standardized dietary conditions.

Previous results from this department showed a weak correlation between the endogenous estrogen levels and histamine turnover in that histamine, MeHi and MeHMAA excretion increased at midcycle (11). The present study seems to support this observation. Stimulation of amenorrhoeic women with gonadotrophin inducing a marked increase in the endogenous estrogen levels resulted in a slight augmentation of histamine and/or metabolite output in 4 of 8 trials. Oral contraceptives (estrogen or gestagen dominated) did not influence histamine turnover significantly. A similar lack of effect was found in the guinea pig (14) whereas a combination of estrogen and progesterone increased histamine output in the mouse (13) and decreased the excretion in the rat (12).

These findings illustrate marked differences in

histamine turnover between different species. It should, however, be born in mind that the oral contraceptives represented a long lasting stimulation whereas the animals were subjected to a hormonal stimulation by single injections. The mechanism behind the steroid induced effect on histamine turnover in the human female is still difficult to explain. It has been shown that exogenous oestrogen on histamine depletion in the uterine tissue (15). It seems, however, not probable that any local alterations within the reproductive organs may be reflected in the urinary output of histamine. Mast cells are presented in nearly all tissues throughout the human body.

Climacteric symptoms are mainly characterized by the occurrence of hot flushes and sweats. The pathogenesis of this syndrome is not well understood. It has been suggested that the vasomotor symptoms should be the result of an interaction between the hypothalamus and the autonomic nervous system brought about by a deficiency of estrogen. Hot flushes in the same region of the brain can be induced by intravenous injection of histamine and it could be speculated that this mechanism might be involved in the pathogenesis of the syndrome.

The present results show that there is no difference in the excretion of histamine and MeHi between postmenopausal women with and without climacteric symptoms. Nor is there a difference in this respect between postmenopausal and

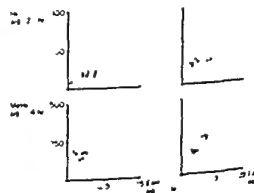


Fig 7 Urinary excretion of histamine (Hi), methylhistamine (MeHi) and estrogen (Estr) in 10 women of postmenopausal age without any symptoms of climacteric. In 10 other women of the same age but with symptoms of hot flushes and/or sweats (climacteric) the same variables were obtained without any treatment. Changes were obtained when the women were treated with 250 µg estradiol (20 µg daily). (Left upper part) subjects 4 and 5.

Table III Urinary excretion of histamine (Hi) and methylhistamine (MeHi) in μg base per 24 hours in 20 premenopausal women (group 3)

The first 10 (A-K) had no symptoms and treatment. The others (L-U) had symptoms of hot flushes and/or sweats and were treated with ethinylestradiol at the time of the second urine collection ($\sim 0.05 \mu\text{g}$ daily)

Subject	Compound	Samples	Treatment	Mean \pm S.E.	Range
A	Hi	0	No	78 ± 3	10-49
	MeHi	70	No	197 ± 16	9-354
	Hi	20	No	27 ± 2	10-46
	MeHi	0	No	202 ± 19	76-364
L	Hi	19	No	22 ± 3	7-59
	MeHi	19	No	192 ± 11	109-289
	Hi	70	Yes	23 ± 4	7-88
	MeHi	0	Yes	195 ± 15	110-247

n (11). It is hard to believe that the probable error distribution of the histamine excretion values for the symptom free women as compared to the values of linearity in patients with symptoms has any clinical significance. It has been observed that patients undergoing cryotherapy for premalignant cervical lesions get temporary hot flushes during thawing of the cervical tissue. Measurement of urinary histamine and MeHi showed an increased excretion for at least four hours following the treatment (18). It seems probable that postmenopausal hot flushes are not elicited by a systemic release of histamine into the circulation.

ACKNOWLEDGEMENTS

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EXPOSURE TO DRUGS AND OTHER POSSIBLY HARMFUL FACTORS DURING THE FIRST TRIMESTER OF PREGNANCY

Comparison of Two Prospective Studies Performed in Sweden 10 Years Apart

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Of 474 women in mid pregnancy interviewed at different hospitals in Sweden were questioned on a number of social and medical items e.g. drug use, contraceptive technique used before pregnancy, exposure to possibly deleterious factors in the environment. They were compared with a similar study made ten years earlier in Sweden. There was little or no difference in the use of iron and/or vitamin preparations, analgesic drugs, antibiotics or endocrine drugs, but a drastic reduction is seen in the use of psychotropic drugs and of histaminic drugs. A marked decrease in frequency of 1 trimester X-ray exposures can be found, but no marked changes in smoking habits. Approximately 18% of the women used contraceptive pills within 6 months of becoming pregnant—3 had used them during early pregnancy, out of 4% (18 women) had used IUD—one became pregnant with a Cu IUD (intra uterine device impregnated with copper).

This type of study can provide some information on the prevalence of relatively common factors, but it must be considerably extended in order to permit an analysis of rare events e.g. use of most drugs.

In recent decades much interest has been shown in the possibility that exogenous factors can damage the developing human embryo, with foetal death or maldevelopment as a result. Various diseases, drugs, smoking, and exposure to environmental pollution are factors with such potential. Various surveillance systems have been introduced for detecting changes in malformation rates and for identifying harmful factors. In order to evaluate such information, knowledge of the prevalence of various possibly harmful factors among pregnant women is needed. Obviously the use of drugs and other exogenous factors can vary considerably between different

countries, in different populations within a country, and with time. Knowledge of such data could help to strengthen or weaken supposed correlations between a specific factor and the occurrence of a specific malformation. Some efforts have been made to collect this type of information—a much quoted study is that by Nora et al. (11) where 240 pregnant American women were questioned during pregnancy, a mean number of 3.7 potentially teratogenic factors was found per woman.

The present study compares the results of two prospective investigations into exogenous factors during pregnancy. Both were performed in Sweden with an approximately 10-year interval. The two studies were designed differently and were performed with somewhat differing aims. This must be borne in mind when comparing the results.

The older study—Study 1—performed in 1963–65, tried to analyse the total population of pregnant women in one city, Malmö, during a specified period. Nearly 6400 pregnancies were studied and all data obtained during the whole of the pregnancy were collected. Some reports from this study have been published (1–5–9).

The more recent study—Study 2—performed in 1973–74, studied a sample of women from ten different hospitals distributed throughout the country. From each woman, information was obtained only once. It referred mainly to events during the first trimester. The information required was thus only part of that sought in Study 1. However, Study 2 raised two points not included in Study 1: (a) concerning contraceptive measures taken during a period of 6 months before pregnancy, (b) co-

Table 1 Specification of items included in questionnaire in Study 2

Date of interview	age of interviewed woman
civil status	
Date of LMP	and number and year of earlier pregnancies and their outcome
Diseases	asthma epilepsy diabetes heart disease
Contraception	technique used during 6 months before pregnancy
Vaccination	within 3 months before and 12 weeks after LMP
<i>Information concerning first trimester</i>	
Place of residence	work outside home possible noxious exposures in home environment or at work
Profession of spouse	Smoking habits Is the pregnancy wanted?
Morning sickness	no very little yes very much
Bleeding since LMP	no single small many small single large many large
Diseases since LMP	
Contacts with contagious diseases	
Operations performed	under complete anaesthesia
X-rays performed	
Use of drugs	including homeopathic drugs kind time when used dosage total amount indication for drug
Other information	in of interest e.g. peculiarities in diet exposure to certain poisons (insecticides pesticides) accidents etc

cerning possible exposures to environmental factors that the woman thought could be harmful to the unborn child. The primary aim of Study 2 was to make a pilot investigation in order to find out whether it is possible in that way to form a sampling network which could supply information on the presence and significance of various exogenous factors with the main stress on drugs that could be noxious to the embryo.

MATERIAL AND METHODS

Study 1 is a prospective study carried out during 1963-65 in the city of Malmö 6376 women (92.3% of all pregnancies during that period) were interviewed when they sought medical advice because of pregnancy outside or inside the hospital. The loss is not random 4.7% were supposed to be included but refused to co-operate 7.9% were not included for undisclosed reasons. There is an over representation among the non included women of those who would go on to have a legal abortion. To make comparisons with Study 2 more meaningful the present

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study comprises only those who continued their nancies to childbirth i.e. 5678 women representing 92.4% of all women who—pregnant during the period—gave birth in Malmö.

Information was obtained mainly through a questionnaire completed during the entire pregnancy by the woman sometimes with the help of the doctor she attended. These data were supplemented with data from other sources mainly from hospital records. Such data concerned first trimester conditions on small extent.

Study 2 was performed during the second half of the first half of 1974. It involved ten different towns fairly well covering the country geographically. Each hospital one or several midwives selected; interviewed approximately 50 women who attend outpatient departments during the second half of the pregnancy. One hospital interviewed only 15 women and the rest between 50 and 67. The actual number of interviews was 474. The actual number of interviews were made varied but total of 474 were performed after the first trimester before childbirth.

Table 1 summarizes the questions in these interviews. Personal instructions were given to all midwives who part in the study in order to explain the aim and to any difficulties of definition. The co-operation from midwives was excellent and we express our sincere thanks to them all for their contribution.

The outcome of the 474 pregnancies was checked could not be identified however. Four had ended in miscarriage (one of them had a hydatidiform mole) comparisons with results from Study 1 these were excluded and only the remaining 464 pregnancies that to have ended in childbirth were used.

RESULTS

1 How representative are the groups studied?

In Study 1 more than 90% of all women who gave birth in Malmö during the period involved part. The excluded cases deviated somewhat from the included group with respect to age and nationality for instance. The less the included group must be regarded as reasonably representative of women that give birth in Malmö during the period involved. However it might differ from the total population of such women in the country as a whole. It is possible to compare the Study 1 sample with the total country with respect to age distribution and civil status as these data are available from the Swedish official statistics. Study 1 group population deviated markedly and significantly with respect to age distribution with an increased percentage of women below 20 years or above 35 years of age ($\chi^2=68.9$ $P<0.001$). The agreement with respect to civil status is good (Fig. 2) $\chi^2=0.78$ N.S.

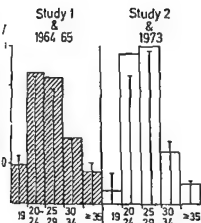


Fig 1 Diagram showing age group distribution—as percentages of the total material—in Study 1 (hatched) and Study 2 (white). The percentages valid for each age group in the total material of all births in the country are marked by vertical lines

Study 2 compared with the total population of childbearing women in all Sweden during 1973 also related with respect to age distribution (Fig 1). The information having been collected at ten sites $\chi^2=16.7$ 0.01 $>P>0.001$. Again the distribution of women below 20 years of age was small. A deviation is found also with respect to status (Fig 2). The percentage of married is lower in the Study 2 group than in the total material ($\chi^2=15.9$ $P<0.001$). The study 2 material probably reflects a mainly urban population. Reasonably large hospitals were included in the sample, four of them situated in large cities. As age and civil status influence drug use and many other similar features, the data obtained must be interpreted carefully.

Changes in age distribution and civil status

Comparison of the 1963-65 data with those of 1974 shows a marked difference in age distribution at childbirth in Sweden: fewer women now have children before 20 years of age (12.5% and 7.5% respectively) and also after the age of 35 (10% and 6% respectively). This difference is further accentuated when the two study samples are compared. A marked reduction has taken place especially for the age group below 20, from 10% in Study 1 to approximately 3% in Study 2. The actual difference in age distribution between the two studies is statistically highly significant ($\chi^2=35.6$ at 4 d.f. $P<0.001$). When mar-

kedly age dependent features of the two studies are compared, a correction for the difference in age distributions should be taken into account.

Fig 2 compares civil status. This comparison is complicated by a shift in matrimonial habits during the decade. As seen in Fig 2, 31% of the women interviewed in Study 2 said they were unmarried but lived together with the infant's father in a marriage situation but without society's formal sanctions. The corresponding group in 1963-65 was probably negligible and was never identified in Study 1. Fig 2 suggests that this civil status is most comparable to an actual marriage situation. The sum of numbers of married couples and couples cohabiting without formal marriage (= unmarried couples) even somewhat exceeds the group married in Study 1.

Another aspect of the socio-medical significance of the different civil status groups can be obtained by studying the incidence of wanted pregnancies in the different groups.

Fig 3 illustrates the findings. In the Study 1 material, approximately 92% of all women that were married when they became pregnant positively stated that their pregnancies were wanted. Naturally, the percentage of wanted pregnancies was much lower in the group of women that were not married at the beginning of their pregnancy, and a considerable percentage of such pregnancies ended in an induced abortion; therefore 67% of women belonging to this category who continued their pregnancy to childbirth had, during pregnancy, reported that the pregnancy was wanted.

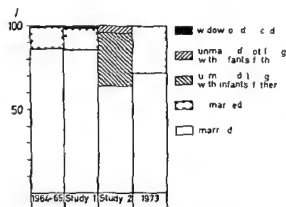


Fig 2 Diagram comparing distribution of civil status groups in Study samples with the total of births in the country

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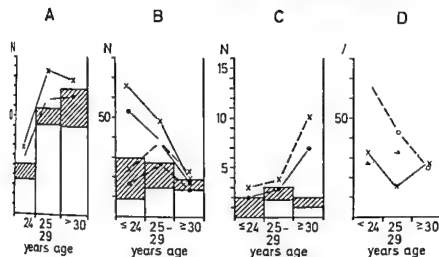


Fig. 5. Diagrams showing found numbers (N) of live births (□) and pregnancies ending with other than live births (hatched columns) and also civil status of different groups in Study 1. A: married women; B: unmarried couples; C: married single. In each Figure the expected numbers from Study 1 are marked: x-x total number of pregnancies; ●-● number of live births from

the group married at LMP in Study 1; dashed lines give the corresponding numbers from the group unmarried at LMP in Study 1. D shows the percentage of pregnancy wastage for each civil status group and each age class: x-x married; o-o unmarried couple; Δ-Δ unmarried single.

group. From the group married at LMP in Study 1 132 were expected from the group unmarried at LMP 80 were expected. In Study 2 39 live births were found—from the group married at LMP 108 were expected and from the group unmarried at LMP 58 were expected. Thus from the standpoint of earlier reproduction this group resembled best the group unmarried in Study 1. Especially if a general reduction in the number of pregnancies is taken into account, such as is seen when the married groups of the two studies are compared.

Fig. 5 compares the expected and found numbers of pregnancies and live births at different civil status and age. For the married group no great differences for different age classes exist in the relation between the two studies. For the group unmarried couples the main deviation is found in the youngest age group (below 20 years)—for the age group above 30 years no actual differences are found between the studies. For the group unmarried single the most marked difference is found for the age group above 30 years.

Fig. 5 also shows the percentage of previous pregnancy wastage in different age groups and for different civil status categories. The most deviating group is that of unmarried couples, especially in

the age group 20 years. Their rate of earlier legal abortion is twice that of the other groups: 6 out of 30 earlier pregnancies against 26 out of 346.

4. Contraceptive technique used before pregnancy

Fig. 6 summarizes data on contraceptive technique used 6 months before pregnancy based on all 474 pregnancies in Study 2. The A diagrams show the percentage of women reporting that no contraception was used. It hardly varies with age, civil status or whether the pregnancy was wanted—the small recorded differences are only indicative, as the material is not very large. A slight increase in this percentage occurs with age and a somewhat higher incidence among women who report a wanted pregnancy than among those who report an unwanted pregnancy.

On average 52% of all women report that they used no contraception during the 6 months preceding the present pregnancy.

Of the 228 women who used some sort of contraception during this period 87 (38%) used the Pill and in 112 cases (49%) the partner had used condoms. 18 (8%) women had an intra uterine device, only 4 (2%) had used a vaginal diaphragm. The other 7 had used various techniques, including

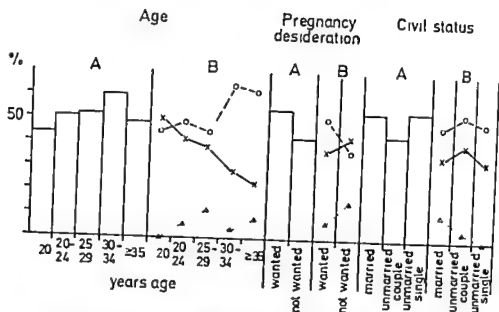


Fig 6 Diagrams showing percentage of women who did not use any contraception for 6 months before becoming pregnant (A diagrams) and its variability with age, pregnancy desideration and civil status. B diagrams show the

percentages using the Pill (x-x), condoms (O--O), IUD (Δ Δ) among women using some contraceptive Division according to same variables.

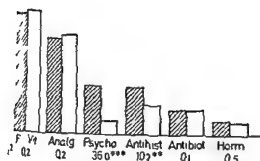
only chemical means and interrupted coitus. One woman using a Cu IUD became pregnant despite the device. The B diagrams in Fig 6 show distribution of the three main contraceptive techniques as a percentage of all women taking some sort of precautions according to age, civil status and whether the pregnancy was wanted. The most obvious variation occurs with age: with increasing age, the use of the Pill decreases with a corresponding rise in the use of the other two techniques, especially of condoms. The three variables—age, wanted pregnancy and civil status—co-vary considerably, but the material is too small to permit a study of the contribution of each source of variation. So far, however, no data argue against the hypothesis that age is the dominating and perhaps the only decisive factor. With that hypothesis, the maximum recorded deviation between found and expected numbers is 2.8—in the group 'unmarried couples, not wanted pregnancy': 5.2 expected and 8 found. This could well be random.

5 Drugs used during the first trimester

Six different groups of drugs were compared in the two studies: iron and/or vitamin preparations, analgesic drugs, antihistamine drugs, mainly used as antiemetics, psychotropic drugs, antibiotics and

chemotherapeutic agents such as sulphonamides and hormones. The percentage of women using or more drugs belonging to each of these categories was known from Study 1 (most of these data published in Refs 6-9) and was determined in Study 2 in the following way. In most instances, the first month during which a specific drug was taken was reported. A drug reportedly used for the first time during the first trimester was called a first trimester drug. Some reports did not explain the state when a drug had been used—this was especially true for iron and/or vitamin preparations and for analgesics.

117 women stated that they had used iron and vitamin preparations during the first trimester; in other cases, the time of drug use was not known. The highest possible number of women using such drugs during the first trimester was thus 182 (39%) and the lowest possible number was 117 (25%). If women reported the use of these drugs during some part of the pregnancy (thus also after the first trimester), therefore 117 (72%) of them had begun drug use during the first trimester. If this figure is valid also for those women that did not state the time of drug use, 72% of 65 women were also first trimester users, making a total of 164 (35%). This estimate is regarded as the most probable one obtainable from Study 2.



7 Drugs used during first trimester expressed as all women who on one or more occasions used drugs belonging to different groups as shown by subscript Fe Vit=iron and/or vitamin preparation analgesic drugs Psycho=psychotropic drugs Antihist=antihistamine drugs Antibiot=antibiotics or antic agents Horm=endocrine preparations Study 1 χ^2 values compare the two studies $01 > P > 0.001$ $P < 0.001$

similar calculation was made for analgesics 89 (19%) reported having used analgesic drugs the first trimester and a further 50 did not when the drug was used making a maximum 30%. The most probable number—calculated described for the iron and vitamin preparation 130 (28%).

Fig 7 compares the first trimester drug use in Study 1 with Study 2. For iron and vitamin preparation analgesics antibiotics and hormones no difference is found between the two studies but a significant reduction in the use of psychotropic drugs and antihistamines can be seen. The latter two types of drugs are used with different frequency according to the desire for pregnancy (6-7). In Study 1 thus 12.7% of women reporting a wanted pregnancy used psychotropic drugs but 23.3% of women reporting an unwanted pregnancy. If these figures are used to estimate the number of drug users in Study 2 where 3 women reported a wanted pregnancy 65 first trimester psychotropic drug users are expected only 16 were found ($\chi^2=36.9$ at 1 d.f. $P < 0.001$). The most frequently used psychotropic drugs in Study 1 were barbiturates meprobamates and chloridazepoxide. In Study 2 the following drugs were recorded: chloridazepoxide (4 women), diazepam (3 women), nitrazepam, barbiturate, lorprothixene, ethchlorvynol, amitriptyline, norfentanyl. In Study 1 72% of all women who later gave

birth complained of morning sickness of varying degree during pregnancy. The corresponding percentage in Study 1 is 71%. The reduced incidence of antihistamine use found in Study 2 compared with Study 1 thus cannot be explained by different frequency of morning sickness complaints. The following antihistamine drugs were used in Study 2: promethazine (18 women), meclizine (10 women), diphenhydramine, prochlorperazine (each one woman), meclizine (3 women), dexbromphenamine, cinnanzine (each one woman)—the three last mentioned drugs are used for allergic complaints. In Study 1 the three most used drugs were promethazine, prochlorperazine and diphenhydramine.

In Study 2 33 women reported that they had been given antibiotics or chemotherapeutic agents during the first trimester—38 different prescriptions had been made. In all except one the indication was an infectious disease—the exception had penicillin as prophylaxis after contact with scarlatina. Table II sums up the diagnoses. 13 patients (about 3%) had urinary tract infections during the first trimester. This is more than was recorded in Study 1 (less than 1%) and is reasonably due to increased awareness of the complications of urinary tract infection during pregnancy leading to earlier detection. Seven of these patients were treated with sulphonamides only 3 with nitrofurantoin and 3 with a combination of both drugs. Ten patients thus had sulphonamides—the rate of first trimester use of this drug in Study 1 (cf 9) was 2.3%. At this rate 11 patients in Study 2 had used the drug.

Ten patients in Study 2 used penicillin during the first trimester mainly because of upper respiratory infections. The expected number from the rate recorded in Study 1 (3.2%) is 15.

In Study 2 endocrine drugs given during the first trimester were reported by 16 women. Three of them had continued with oral contraceptive pills during the first part of the pregnancy. Eight had gestagens. This figure compares well with the 2% found in Study 1. Four of the 8 women who took gestagens reported bleeding during early pregnancy the other 4 had previously had one or more miscarriages. Seven of the 8 women were treated at one and the same hospital from where 54 reports were obtained. Among them 10 had an earlier miscarriage (one had had two) and 4 of the 10 had gestagens. A further 8 had bleeding during pregnancy 3 of them had taken gestagens. Among the 410

Table II Infectious diseases reported in Study 2 during the first trimester and use of antibiotic (or chemotherapeutic) drugs

Diagnosis	No of patients	No using antibiotics	No of diagnoses
Common cold	63	9	64
Otitis sinusitis	3	1	3
Tonsillitis	8	1	8
Influenza	22	1	22
Virus disease	1	0	1
Subtotal all upper respiratory tract infections	97	22	98
Enteritis	5	2	5
Urinary tract infections	13	13	13
Vaginitis	1	0	1
Skin infection	1	1	1
Total	110	33	118

women interviewed at other hospitals. 54 had one or more earlier miscarriages—none had taken gestagens. 65 reported bleeding during pregnancy—only one had taken gestagens. She had repeated slight bleeding but no previous miscarriage.

In Study 1 16.6% of women who later gave birth reported bleeding during early pregnancy (cf. 8) and almost the same incidence was found in Study 2. 73 women (16.6%). Of these only 8 had the formal diagnosis of threatened abortion. Together these 8 women had three earlier pregnancies, two of which ended in miscarriages. Out of the total material only 19% of earlier pregnancies ended in miscarriage.

Smoking, X rays and vaccinations during pregnancy

Fig. 8 compares the two studies with respect to the occurrence of smoking, X ray investigations and vaccinations during the first trimester.

Smoking habits have changed little. In Study 1 (cf. 5) 41.6% of women that later gave birth smoked during pregnancy—in Study 2 this would mean 193/187 were found. Among the smokers a shift had occurred towards more cigarettes smoked per day (cf. Fig. 8). If the three groups are compared between the two studies (non smokers, smoking less than 10 and smoking more than 10 cigs/day) the difference appears significant ($\chi^2=9.8$ at 2 d.f. $0.01 > P > 0.001$).

The incidence of diagnostic X ray investigation performed during the first trimester is lower in Study 2 than in Study 1. In Study 1 6% of the women had a dental X ray performed during this period—this would correspond to 78 women in Study 2 but only 9 were found ($\chi^2=17.9$ at 1 d.f. $P < 0.001$). Chest X rays had been taken in 5% in Study 1 this would correspond to 23 women in Study 2—only 8 were found ($\chi^2=9.8$ at 1 d.f. $0.01 > P > 0.001$).

The most common vaccination registered in Study 2 was for smallpox. Seven women had been vaccinated during the first trimester and a further 1 within 3 months before their pregnancies. In Study 1 1.8% of the women had been vaccinated for smallpox during the first trimester this would correspond to 8 women in Study 2.

7 Miscellaneous

In Study 2 the women were asked to mention exposures during the first trimester—at work or home—to factors that could be thought harmful to the unborn child and also to peculiarities in the diet. 222 women (47%) mentioned such factors and a total of 269 exposures were detailed together with 9 dietary peculiarities. Table III summarizes the former data arbitrarily grouped. A total of 70% exposures at work were thus mentioned and 67% exposures in the home environment or otherwise outside the working place. One large group is exposures to contagious diseases during work, work in hospitals or other medical work, service jobs leading to frequent contact with a large number of people and work with children in schools etc. Among the many other items mentioned is exposure to paints and organic solvents—35 women (7.7%) mentioned this. Five women reported work operating theatres which means possible exposure to leaking volatile anaesthetics. X ray work was reported by 11 women and work with radioactive isotopes by 4. Among the dietary peculiarities blighted potatoes was mentioned once.

The number of recorded exposures could to some extent be related to the interview technique. When the number of women giving information on the points was compared between the different hospitals differences were found. The lowest frequency recorded was 15 out of 56 interviewed (27%) and the highest 34 out of 50 interviewed (68%). A χ^2 test for heterogeneity in reporting frequency between the hospitals gives $\chi^2=30.5$ at 9 d.f. $P < 0.001$.

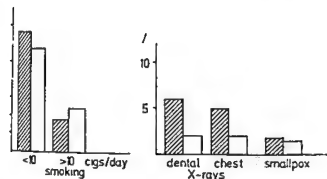


Fig 8 Diagrams showing percentages of women who smoked or were exposed to first trimester X rays or first trimester smallpox vaccination (▨) data from Study 1 (□) from Study 2

Malformation and perinatal death

Study 2

Material in Study 2 is too small to give meaningful information on specific factors related to malformations or perinatal death. Table IV summarizes the outcome of the pregnancies with stress on abnormalities and infant death. This information is based on the neonatal records submitted to birth registration (sometimes supplemented with data from hospital records) and is therefore probably incomplete. It cannot be directly compared with data from Study 1 (1) as those records on malformations were more detailed. There were remarkable first trimester occurrences in the group that ended with abnormal births in

2

DISCUSSION

If the human embryo is exposed to various possibly harmful influences of exogenous origin. Some have a definitely injurious effect, e.g. maternal smoking (5-10) the deleterious effect of other factors is hypothetical. A comparison of the effect of exposures in the two studies reveals differences. Smoking has not markedly decreased during the intervening ten years between the two samples if they represent the smoking habits of the total Swedish population of pregnant women. Perhaps Study 1 overestimated the smoking habits in the mid sixties, as this sample was from a purely urban population. The research efforts to reduce smoking by information apparently had little effect on this important group of pregnant women. No definite harmful effect on the embryo can be ascribed to analgesic drugs (cf. Discussion in 9)

when used in moderation. None the less various possibly deleterious effects, including teratogenicity, have been widely published even in the lay press. Despite this, no decrease in frequency in the use of analgesic drugs has occurred.

By contrast, a definite decrease in exposure to psychotropic drugs and antihistamine drugs can be recorded. The same is true of X-ray exposures. All are more or less governed by medical advice, and the (unproved) possibility of harmful effects on the embryo can have contributed to an increased caution in their use in first trimester pregnancy. There has been a general restriction in the use of psychotropic drugs in Sweden during this period. No decrease of a comparable size in the use of antibiotic drugs or endocrine preparations can be found. The latter group, however, demonstrates the inherent risk in such comparisons. Out of the ten hospitals studied, one issued nearly all prescriptions for gestagens. If that hospital had not taken part in the study, it could easily have been concluded that such drugs are no longer used during pregnancy to prevent a threatened abortion.

This raises the issue of how representative the samples are. The Study 1 sample can be regarded as representative of the population of pregnant women in Malmö during this period, as 92% were included, but it deviates with respect to age distribution from the total population of child-bearing women in Sweden. Data on drug use obtained from the Study 1 sample can markedly deviate from the general drug use—and it most probably does! In Study 2, an effort was made to overcome this source of error by distributing the interviews among ten different hospitals throughout the country. The above described example with gestagens shows that this effort can easily fall short of the ideal. The skew

Table III Exposures of various exogenous factors mentioned by interviewed women in Study 2 (all interviews)

Group	Subgroups	Exposures		Total of exp women
		At work	At home	
Exposure to contagious diseases	Work in medical care	59		125
	Service jobs	37		
	Work in child care	28		
	Work with animals	1		
Exposure to possibly harmful chemicals	Paints, solubles	14	21	63
	Insecticides, pesticides etc		12	
	Laboratory chemicals	8		
	Hair sprays etc	3	7	
	Volatile anaesthetics	5		
	Solder smoke	1		
Exposure to a generally poor milieu	Car exhausts	2	20	76
	Poor ventilation and noise	4		
	Work in smoky rooms	1		
Physical strain or trauma	Heavy lifting	11		18
	Violence, accidents	1	4	
	Animal bites		2	
Psychological trauma	Stress at work	17		18
	Grief etc		6	
Exposure to ionizing irradiation	Work with X ray	11		
	Work with radioactive isotopes	4		
Totals		203	67	217

distribution found in age and civil status is also a bias. Its origin can lie in the selection of hospitals—leading for example to an over representation of urban populations—but it can also be the result of an unintended selection of women for interview. Whatever bias is obtained in Study 2, the recorded data can give a rough estimate of the efficiency of this type of data collection system.

If the data are collected to identify women who used specific drugs during early pregnancy in order to search for teratogenicity, the study must in most instances be considerably extended. Except for a few quite common drugs (e.g. chlorthalidopoxide, diazepam, meclizine, promethazine) only isolated records of drug use were found in this sample of 474 persons. To obtain material large enough to make analysis for teratogenic effects meaningful, possibly ten times as much data is needed.

If the two study samples are at least reasonably representative of the drug use by pregnant women in Sweden during two periods ten years apart, some comments can be made on changes in drug use. The use of psychotropic drugs during the first trimester has been markedly reduced, perhaps to a quarter. If

the figures obtained in Study 1 are representative of the entire country, possibly 15 000 women used such drugs during the early part of pregnancy each year in the mid sixties. If the Study 2 figures are also representative of the 1973 situation, the number has been reduced to possibly 4 000, an elimination of perhaps 10 000 first trimester ex-

Table IV Summary of offspring in the 464 pregnancies

Total number 464
Live born 458
Stillborn 6

Abnormalities recorded 15

Down's syndrome	1
Anencephaly	1
Hypopspadias	1
Syndactyly	1
Persistent ductus arteriosus	1
Heart murmur (no final diagnosis)	1
Pes equino-varus	1
Pes calcaneo valgus	3
Proluxation of the hip	1
Preauricular appendix	1
Undescended testis	1
Imperforate hymen	1
Penis recurvatum	1

to psychotropic drugs has in no detectable way reduced the incidence of malformed infants in the country (cf 2). The reduction in anti-stomach use is less marked but—calculated in the same way—could perhaps represent 5000 exposures.

Little is known about the possibly harmful effect of chemicals in the everyday life on the development of the human embryo. The information gathered in Study 2 and detailed in Table III on such matters probably mainly reflects the apprehensions and suspicions held by the interviewer and the interviewee. This is further supported by the significantly variable amount of information obtained by different interviewers in different hospitals.

Some of the recorded factors have been more seriously discussed in the scientific literature: teratogenesis e.g. ionizing irradiation (cf 12), volatile anaesthetics (3), organic solvents (4), blight on potatoes (13). Others have been much discussed locally e.g. insecticides and pesticides, others carry a long standing bad reputation among laymen e.g. heavy lifting and psychological stress.

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SERUM HUMAN PLACENTAL LACTOGEN (HPL) LEVELS IN PATIENTS WITH INTACT HYDATIDIFORM MOLE

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Serum human placental lactogen (HPL) levels in many cases of intact hydatidiform mole were measured by immunoassay. The HPL values were generally lower than normal pregnancies of the corresponding period of gestation. However, normal and occasionally higher than normal values were observed in a few cases. Serum HPL measurement is of some clinical use in the diagnosis of hydatidiform mole. When combined with human chorionic gonadotropin (HCG), a low HPL/HCG ratio for the corresponding period of amenorrhoea is a useful index in the diagnosis of hydatidiform mole.

The production of human chorionic gonadotropin and human placental lactogen are inherent properties of the trophoblast in all its forms—normal placenta, hydatidiform mole and choriocarcinoma. Human placental lactogen (HPL) is produced by the cytotrophoblastic giant cells of the placental syncytiotrophoblast (14). The presence of HPL in the sera of patients with hydatidiform mole is of interest as it provides a possible endocrine index of following the course of these tumours.

Earlier studies on the level of HPL in hydatidiform mole have produced contradictory results. Grumbach and Kaplan (5) first reported high levels of HPL in a case of hydatidiform mole. Similar low HPL values in hydatidiform mole were reported in other studies by Frantz et al (2), Samraan et al (11), Yen et al (10), Saxena et al (13), Chough (1) and Sato et al (3). Values were thought to be invariably low, often 10–100 times below the normal expected values for the corresponding gestational age. In an early normal pregnancy of less than 10 weeks gestational age, subsequently Goldstein (4) reported HPL levels in 94 cases of unevacuated molar pregnancies. He noted a wide range of serum HPL

values from those consistent with a normal pregnancy to 10–100 times below the normal expected values for the corresponding gestational age (See Table I).

The present study was done to evaluate further the significance of the observed abnormality in serum HPL in patients with intact molar pregnancies. The potential value of HPL as a useful adjunct in the endocrine diagnosis of molar pregnancy was investigated. A subsequent study will deal with sequential assay of serum HPL during the course of choriocarcinoma to determine its usefulness as an index in the management of the disease.

MATERIAL AND METHODS

All cases of hydatidiform mole were seen by the same mole team. Only cases of intact molar pregnancy were included in this study. The criteria of selection were (a) There must be no passage of molar vesicles per vaginam. Spotting per vaginam was acceptable since the majority

Table I Cumulative data on serum HPL in patients with hydatidiform mole

Authors	No of cases	HPL level		
		Low	Normal	High
1 Grumbach (1964)	1			+
2 Frantz (1965)	2	+		
3 Samraan (1966)	4	+		
4 Yen (1968)	1	+		
5 Saxena (1968)	7	+		
6 Goldstein (1971)	94	+	+	
7 Chough (1973)	9	+		
8 Sato (1973)	20	+		
9 Lim (1974)	40	+	1 case	1 case

Table II Serum HPL and serum HCG in patients with intact hydatidiform mole

No	Amenorrhoea (weeks)	Uterine size (weeks)	Serum HPL (μ g/ml)	Serum HCG (IU/ml)	HPL (ng/ml)		Sequelae
					HCG (IU/ml)		
1	16	24	1 740	370	5 40		Chorio Ca
2	23	18	0 270	480	0 56		Chorio Ca
3	14	16	0 097	60	1 67		Chorio Ca
4	12	18	0 090	480	0 19		Chorio Ca
5	35	22	0 180	60	3 00		
	36	22	0 170	740	0 71		Chorio Ca
6	13	18	0 270	240	1 13		Chorio Ca
7	12	18	0 200	160	1 25		Chorio Ca
8	14	24	0 074	960	0 08		Chorio Ca
9	24	24	0 300	1 280	0 73		Chorio Ca
10	12	18	0 062	640	0 10		Benign
11	12	16	0 110	370	0 34		
	13	16	0 180	3 0	0 56		Benign
12	14	19	0 140	640	0 77		Benign
13	31	76	0 240	40	6 00		Benign
14	14	20	0 200	480	0 42		Benign
15	18	18	0 0 0	480	0 04		Benign
16	19	20	0 130	480	0 27		Benign
17	9	12	0 030	1 80	0 02		Benign
18	12	16	0 013	170	0 11		
	13	17	0 036	320	0 11		Benign
	15	18	0 040	30	1 33		
19	16	24	0 130	480	0 77		Benign
0	19	24	0 170	3 0	0 38		Chorio Ca
21	17	24	0 010	160	0 06		Benign
22	12	24	0 350	1 280	0 77		Benign
	8	12	0 046	960	0 05		
23	13	16	0 080	480	0 17		Chorio Ca
	15	20	0 180	960	0 19		
24	11	18	0 047	480	0 10		
	12	18	0 060	3 0	0 19		Chorio Ca
	13	18	0 073	640	0 11		
25	18	14	0 010	20	0 50		Chorio Ca
26	18	24	0 500	1 0	4 17		Benign
27	12	20	0 080	3 840	0 07		Benign
28	14	20	0 100	480	0 71		Benign
29	15	20	0 110	480	0 73		Benign
30	18	28	0 230	300	0 77		Benign
31	12	22	0 080	1 280	0 06		Benign
32	19	24	0 010	1 970	0 01		Benign
33	24	18	0 180	10	18 00		Benign
	19	0	0 110	640	0 17		Benign
	17	20	0 010	15	0 67		Benign
6	13	18	0 032	240	0 13		Benign
37	14	24	0 058	1 970	0 03		Benign
38	26	77	<0 005	160	0 03		Chorio Ca
39	16	24	0 100	170	0 80		Benign
40	15	70	0 011	960	0 01		Benign

of our patients with hydatidiform mole present with abnormal uterine bleeding (18)

(b) All blood samples for assay were collected from cases just prior to the elective termination of the hydatidiform mole

(c) The final diagnosis of molar pregnancy was based on histologic examinations of the molar vesicles passed per vaginum or from material obtained at hysterectomy

Forty patients were available for this study single samples were obtained from each patient. However 6 patients had more than one sample drawn during the course

of investigations to confirm the diagnosis of molar pregnancy. The final number of samples available were 48. gestational age of the hydatidiform mole ranged from 8 weeks. Ten ml of venous blood was collected prior to elective termination of the molar pregnancy. The serum was separated within two hours of collection and stored at -20°C until ready for assay.

Venous blood was similarly collected from 36 patients with normal pregnancy between 5-40 weeks. Only a sample from each patient was taken to give a range of base line HPL in normal pregnancy.

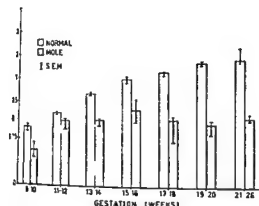


Fig 1 Mean \pm S.E.M. Serum HPL in normal and molar pregnancy

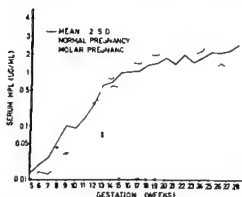


Fig 2 Serum HPL levels in molar pregnancy

selection of patients were as laid out in a previous study by the same authors (9). The number of assays per gestational week ranged from 4-70. Serum HCG was measured by the hemagglutination-inhibition method of Wide (19) as modified by Teoh (17). Serum HPL was measured by radioimmunoassay method previously described by Lim et al (9). This is a highly sensitive and rapid method for measuring serum HPL, and a modification of the method of Letchworth et al (8). The HPL standard was from Amersham radiochemical centre UK. The sensitivity of the assay was such that doses of less than 0.005 μ g/ml cannot be detected.

RESULTS

Table II illustrates the endocrine and clinical profile of the 40 cases. Table III shows the mean \pm

S.E.M. HPL values in patients with intact hydatidiform mole when compared to the levels found in normal pregnancies of corresponding gestational age. The results indicate that HPL levels were significantly lower in cases of intact molar pregnancy than in patients with normal pregnancies of similar gestation. This is also depicted as a histogram in Fig 1. The HPL values in intact molar pregnancies ranged from 2-30 times lower than normal pregnancies of the corresponding period of gestation. However normal and occasional higher than normal values were observed for the corresponding period of amenorrhoea (Table IV).

Fig 2 shows the serum HPL values for the intact hydatidiform moles superimposed on the sectional

Table III Serum HPL and HCG levels in normal and molar pregnancies

Type of pregnancy	Gestation (weeks)	No. of cases	Mean HCG (IU/ml)	HPL (μ g/ml)		
				Range	Mean \pm S.E.M.	Difference
Normal	8-10	24	100	0.038-0.147	0.080 \pm 0.007	$p < 0.0005$
Mole		2	1.0	0.030-0.046	0.038 \pm 0.008	highly significant
Normal	11-12	18	125	0.151-0.420	0.286 \pm 0.019	$p < 0.0005$
Mole		10	892	0.013-0.350	0.109 \pm 0.031	highly significant
Normal	13-14	8	95	0.540-0.750	0.686 \pm 0.027	$p < 0.0005$
Mole		12	565	0.032-0.770	0.115 \pm 0.02	highly significant
Normal	15-16	13	60	0.700-1.40	1.00 \pm 0.065	$p < 0.0005$
Mole		7	478	0.011-1.740	0.330 \pm 0.236	highly significant
Normal	17-18	17	45	0.740-2.000	1.43 \pm 0.072	$p < 0.0005$
Mole		6	182	0.010-0.500	0.130 \pm 0.082	highly significant
Normal	19-20	18	40	1.170-2.340	1.674 \pm 0.069	$p < 0.0005$
Mole		4	840	0.010-0.130	0.092 \pm 0.027	highly significant
Normal	21-26	69	25	1.0-0.350	1.876 \pm 0.056	$p < 0.0005$
Mole		4	483	0.005-0.300	0.189 \pm 0.067	ant

Table IV Range of serum HPL levels in intact molar pregnancies

Values	Range	No of cases	Case number
(A) Very low	31-100 times lower than normal	9	Cases 15 17 18 21 25 31 35 38 40
(B) Low	2-30 times lower than normal	29	The remaining 29 cases excluding (A) (C) (D)
(C) Normal	-	1	Case 22
(D) High	-	1	Case 1

normal HPL curve based on 356 normal pregnancies

Serum HPL and uterine size There was no significant correlation between HPL values and uterine size (coefficient of correlation $r=+0.3$). This is represented in Fig 3.

Serum HPL and serum HCG No significant correlation exists between serum HPL and serum HCG for any given case of hydatidiform mole (coefficient of correlation $r=0.00017$). This is represented in Fig 4.

Ratio of HPL to HCG (ng/ml IU/ml) In normal pregnancy good correlation is seen between this ratio and gestation period. The base line HPL and HCG values were obtained from local series of Lim et al (9). Low ratio values were seen in all cases of hydatidiform mole. This is depicted in Fig 5.

Outcome of molar pregnancy

An attempt to predict the final outcome of the molar pregnancy was attempted. This was done by expressing as an endocrine ratio of HPL/HCG for given case of molar pregnancy. No correlation

ship was found. This is to be expected since there is no significant correlation between serum HCG and serum HPL for any given case of hydatidiform mole. Fig 6 illustrates this point.

DISCUSSION

HPL is produced by the trophoblast both *in vivo* and *in vitro* (10, 11). Studies with tissue culture indicate that the production of HCG is the most primitive property of the trophoblast in the undifferentiated stem cell state. HPL is produced primarily in the differentiated trophoblast; it is only present in small amounts in the undifferentiated stem cell (10).

Original work by Saxena et al (13) shows that HPL levels in molar pregnancies were always significantly low and that there was no overlap with normal values. Josimovich (7) suggested that molar pregnancy could be diagnosed on the basis of low serum HPL. The present authors do not wholly agree with this. Though the majority of values were

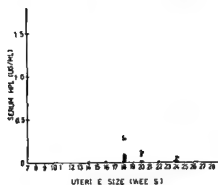


Fig 3 Relationship between serum HPL and uterine size in intact molar pregnancy

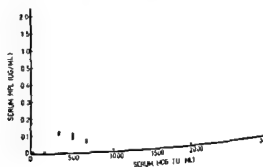


Fig 4 Relationship between serum HPL and serum HCG in intact molar pregnancies

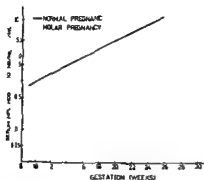


Fig 5 Ratio of serum HPL/HCG in normal and molar pregnancies

low values were sometimes within the normal pregnancy range before the mole aborted and occasionally fairly high values were present as shown in this study. Previous low values as reported may be obtained from patients who had already aborted

or vesicles. An aborted but unevacuated pregnancy is quite a different clinical entity to an intact molar pregnancy. It may be that low levels of HPL as hitherto reported may be due to the passage of molar vesicles with decrease trophoblastic mass. Good correlation between serum HPL and trophoblastic mass have been reported by Celenkew (15) and Seppala et al (16). In vitro cell culture experiments of HPL content in molar tissue

Sato (12) showed that HPL content tend to be lower in the third month of molar pregnancy when compared with similar gestation of normal pregnancy. However, no difference was observed between the two in the fourth month of pregnancy.

Abnormal uterine bleeding was the presenting symptom in all the forty cases under study. It is possible that repeated recurrent bleeding with resultant destruction of trophoblastic tissue may lead to reduced levels of circulating HPL (3, 21). Thus the extent and severity of bleeding per vaginum may also be reflected in the lower levels of HPL in cases of hydatidiform mole.

We do not agree that the level of serum HPL in a hydatidiform mole is a reflection of the length of gestation.

(4) Table III shows that the highest mean HPL level in hydatidiform mole occurred in the 5-16 gestational week (0.33 $\mu\text{g/ml}$). While mean HPL in later gestational weeks were much lower (17-18 weeks = 0.13 $\mu\text{g/ml}$, 19-20 weeks = 0.092 $\mu\text{g/ml}$, 21-26 weeks = 0.189 $\mu\text{g/ml}$).

Goldstein (4) proposed that the level of HPL is

inversely proportional to the degree of malignancy of the hydatidiform mole. This was not found to be so in the present study. Neither the level of HPL per se nor the ratio of HPL/HCG was any indicator of the final outcome of the molar pregnancy. The histologic grouping employed by Goldstein according to the criteria of Hertig & Sheldon (6) have been noted to be unreliable by Tow (18).

In conclusion, serum HPL in intact hydatidiform mole was not found to be invariably low as previously reported. Values ranging from very low to those higher than the corresponding gestational age of normal pregnancy were found. Serum HPL level per se is of some clinical use as an endocrine index in the diagnosis of hydatidiform mole. But when combined with HCG, a low HPL/HCG ratio for the corresponding period of amenorrhoea is a useful index in the diagnosis of hydatidiform mole.

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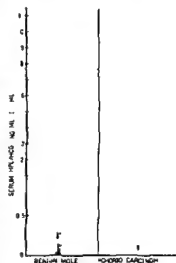


Fig 6 Ratio of HPL/HCG and outcome of molar pregnancy

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CHANGES IN AMNIOTIC FLUID PHOSPHOLIPIDS ON TREATMENT WITH GLUCOCORTICOIDS TO PREVENT RESPIRATORY DISTRESS SYNDROME

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Amniotic fluid was obtained by transabdominal
sis in 51 women in the 29th-36th week of
pregnancy. The lecithin/sphingomyelin ratio was de-
termined in 28 patients; the ratio was <2.2 . Beta-
methasone was given for three days to 14 of them; the rest
served as controls. On the fourth day a second amnio-
centesis was performed on all patients. The lecithin/
sphingomyelin ratio rose to a value >2.2 in 4 of the
14 in the betamethasone treated group and in one of the
14 in the control group.

The percentage of palmitic acid in the lecithin
fraction increased concomitantly with increasing lecithin/sphingo-

myelin ratio. These results suggest that glucocorticoid
treatment of fetal pulmonary maturation may be
reflected in the amniotic fluid as changes in its phospholipid
composition. The lecithin/sphingomyelin ratio was found
to be >2.2 in 23 patients at the first amniocentesis. In such
cases treatment with glucocorticoids can be avoided.

appropriate therapeutic means of accelerating the
maturation of the fetal lungs.

In recent years many investigators have tried to
find pharmacological means to accelerate pulmo-
nary maturity. For this purpose several drugs such
as heroin (15, 31), thyroxine (26, 33), isoxsuprine (18,
34), methylxanthine (23), nitodrin (4), bromhexine
metabolite VIII (8, 24) and oestrogen (30) have
been suggested. The effect of glucocorticoids has
been most extensively studied. Thus accelerated
lung maturation with enhancement of surfactant
production and secretion has been demonstrated in
animal experiments (9, 20, 21, 25), in tissue culture
studies (11, 13, 28, 29, 32) and in clinical studies (6,
7, 14, 22).

The present investigation is a controlled clinical
trial to evaluate antepartum glucocorticoid
treatment for preventing RDS as judged from
changes in the composition of amniotic fluid phos-
pholipids.

MATERIAL AND METHODS

Amniotic fluid was obtained by transabdominal amnio-
centesis in 51 women in the 29th-36th week of pregnancy.
All were in hospital because of complications in the form of
threatened premature labour ($n=28$), suspected placental
insufficiency ($n=6$), Rh immunization ($n=2$), suspected abruptio placentae ($n=$
1), stillbirth ($n=1$), malignant melanoma ($n=$
1), lymphoma ($n=2$), and increased concentration of
fibrinogen in amniotic fluid. Degradation products (FDI)
of amniotic fluid lecithin/sphingomyelin ratio
on the day of amniocentesis. Where the
lecithin/sphingomyelin ratio was <2.2 every second pa-
tient received betamethasone disodium phos-
phate acting betamethasone acetate (C

Respiratory distress syndrome (RDS)—character-
ized by expiratory grunting, tachypnea persisting
after the first hour of life, marked and persistent
subcostal and intercostal recession during
respiration, cyanosis and characteristic radiographic
signs of diffuse reticulogranular mottling in the
lung fields and an air bronchogram (17)—is as-
sociated with deficiency of pulmonary surfactant (2).
Premature infants are most susceptible to develop
RDS, but the condition has also been
observed in infants born at term. Dipalmitoyllecithin
is an important component of the pulmonary
surfactant (19). It is secreted into the alveoli during
respiratory movements; it reaches the amniotic
fluid (3). Analysis of amniotic fluid phospholipids
is possible to estimate pulmonary maturity
and the risk of the newborn developing RDS (10, 16).
This is of limited practical value without

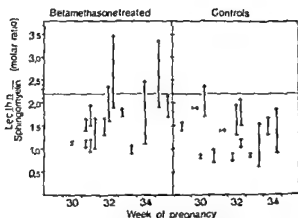


Fig 1 Individual lecithin/sphingomyelin ratios in amniotic fluid obtained by amniocentesis with 4 days interval. The base of the arrow indicates the first value and the head the second

Schering corp) 1 mg daily for 3 days. On the fourth day all patients underwent a second amniocentesis with subsequent analysis of the phospholipids. The analytical results were not disclosed to the obstetricians who had to treat the patients.

Analytical

The amniotic fluid samples were centrifuged at 1000 g for 15 min and the supernatants were decanted. The lipids of the supernatants were extracted with chloroform-methanol 2:1 (v/v) and the phospholipids were separated by thin layer chromatography (27). The lecithin and sphingomyelin fractions were isolated quantitatively (1) and their P-content was determined spectrophotometrically. The fatty acid composition of the lecithin fraction was determined by gas-liquid chromatography.

RESULTS

The lecithin/sphingomyelin ratios of the amniotic fluid samples obtained at the first amniocentesis

were < 2.2 in 28 patients. Celestona Bifas[®] was given to 14 of them. In the amniotic fluid obtained at second amniocentesis the lecithin/sphingomyelin ratio exceeded the value of 2.2 in 4 of the patients: the betamethasone treated group and in one of the controls (Fig. 1). The percentage of palmitic acid in the lecithin increased concomitantly with increased lecithin/sphingomyelin ratio (Table I). Table II gives individual analytical and clinical data of the betamethasone treated patients and Table III that of the controls. No infant developed RDS. The 23 patients that had an initial lecithin/sphingomyelin ratio > 2.2 were in the 31st–36th week of pregnancy. The complications of these pregnancies were: imminent premature labour ($n=12$), Rh immunization ($n=5$), hypoaemia ($n=2$), suspected placental insufficiency ($n=1$), suspected abruptio placentae ($n=1$), malignant lymphoma ($n=2$). No infant developed RDS.

DISCUSSION

In a clinical trial Liggins et al. (22) demonstrated significantly lower incidence of RDS in premature infants delivered after betamethasone treatment of the mother. In a few cases the lecithin/sphingomyelin ratio of amniotic fluid samples obtained before treatment and after treatment was determined but no figures were given. The trial was repeated by Fargier et al. (11) who confirmed the results of Liggins et al. (22). Spellacy et al. (30) treated patients in the 28th–32nd week of pregnancy with betamethasone for two weeks. The mean difference between the lecithin/sphingomyelin ratio before treatment and after treatment was higher in these patients than in a control group. Caspi et al. (6) reported a case of premature rupture of the membranes

Table 1 Fatty acid composition of amniotic fluid lecithin in five cases with lecithin/sphingomyelin ≥ 2.2 after treatment with betamethasone

The fatty acids are designated by (number of carbon atoms) (number of double bonds)

	Pre treatment						Lec /sph (molar ratio)	Post treatment						Lec /sph (molar ratio)
	Fatty acid (mol percent)							Fatty acid (mol percent)						
	14 0	16 0	16 1	18 0	18 1			14 0	16 0	16 1	18 0	18 1		
1	19	71.8	2.2	11.0	13.1	1.9		2.4	86.4	0.7	8.9	1.5		3.4
2	16	67.8	1.5	15.4	13.7	1.1		1.2	85.7	trace	9.3	3.8		2.5
3	13	72.0	7.7	10.6	8.4	1.6		0.5	81.5	1.6	9.4	7.1		2.4
4	36	70.5	4.4	13.3	8.2	1.6		2.8	76.4	1.1	11.9	7.8		3.5
5	21	67.6	2.0	16.7	11.8	1.9		3.2	84.2	1.1	9.9	1.6		

Table II Individual data of betamethasone treated patients

Case no	Complication of pregnancy	Gest age (weeks)	Lec /sph (molar ratio)		Gest age at delivery (weeks)	Birth weight (g)
			Amn c 1	Amn c 2		
1	Susp plac insuff	30	1.1	1.2	35	1 540
2	Impending premature labour	31	1.2	1.0	40	3 300
3	Impending premature labour	31	1.4	1.7	36	2 190
4	Susp plac insuff	31	1.2	0.9	34	1 270
5	Susp plac insuff	31	1.5	2.0	36	230
6	Impending premature labour	31	1.0	1.7	37	2 800
7	Impending premature labour	32	1.3	1.7	40	2 630
8	Impending premature labour	32	1.6	2.4	37	2 820
9	Impending premature labour	32	1.9	2.5	40(?)	2 300
10	Impending premature labour	33	1.7	1.9	38	2 830
11	Rh immunization	33	0.9	1.1	37	2 500
12	Impending premature labour	34	1.1	2.5	40	3 850
13	Susp plac insuff	35	1.6	2.2	38	2 450
14	Impending premature labour	35	1.9	3.4	36	1 940

The lecithin/sphingomyelin ratio remained unchanged for 8 days but rose sharply after treatment with dexamethasone for three days. Recently Caspi et al (7) reported a rise in amniotic fluid lecithin/sphingomyelin ratios to values over 2 after dexamethasone administration. Dexamethasone, however, was given in various dosages and administered in different ways; moreover the interval between pre- and posttreatment amniotic fluid samples varied from case to case and they did not include any controls.

In the present investigation the amniotic fluid samples were obtained identically in both the betamethasone treated group and in the controls. Betamethasone was administered 1 mg in the same

dosage to all treated patients and the second sample of amniotic fluid was obtained after the same interval.

The results thus show that the amniotic fluid lecithin/sphingomyelin ratio exceeded the value of 2.2 in 4 of the betamethasone treated patients and in one of the controls.

The value of 2.2 was chosen on the basis of an earlier prospective study (10) using the same analytical technique. In that study 9 infants developed RDS. In 7 of these cases the lecithin/sphingomyelin ratio in amniotic fluid obtained 0.5-5 hours before delivery was <2.0. The other 2 had a value of 2.2. The lowest value hitherto observed with our analytical method among newborns not developing RDS

Table III Individual data of controls

Case no	Complication of pregnancy	Gest age (weeks)	Lec /sph (molar ratio)		Gest age at delivery (weeks)	Birth weight (g)
			Amn c 1	Amn c 2		
1	Rh immunization	29	1.4	1.6	37	2 770
2	Rh immunization	30	1.9	1.9	37	2 760
3	Impending premature labour	30	0.8	0.9	39	3 860
4	Impending premature labour	30	1.7	2.4	36	2 140
5	Impending premature labour	31	0.7	1.0	42	4 700
6	Impending premature labour	31	1.4	1.4	34	2 020
7	Diabetes mellitus	32	0.9	0.7	39	2 170
8	Susp plac insuff	32	1.3	2.0	35	1 930
9	Increased conc of serum FDP	32	1.2	1.0	40	2 400
10	Impending premature labour	32	1.5	2.1	38	2 960
11	Impending premature labour	33	0.9	0.8	38	2 360
12	Susp abrupt plac	33	0.6	1.6	37	2 610
13	Malignant melanoma	34	1.3	1.7	36	2 210
14	Impending premature labour	34	0.9	1.9	4 (?)	3 910

is 2.0. Probably a borderline group exists with a lecithin/sphingomyelin ratio between 2.0 and 2.5.

The concomitant increase in the percentage of palmitic acid in the lecithin indicates that betamethasone enhanced the synthesis and release of dipalmitoyllecithin. Recently we found evidence both *in vitro* (12, 13) and *in vivo* (11) of accelerated production of dipalmitoyllecithin in human fetal lung tissue after administration of glucocorticoids. The effects were observed as early as the second trimester of pregnancy and within 2 days of treatment. This agrees with observations by Liggins et al. (22) that premature infants delivered 48 hours or later after betamethasone treatment of the mother had a lower incidence of RDS.

It is notable that betamethasone failed to increase the lecithin/sphingomyelin ratio to a value over 2.2 in several cases. This could be because betamethasone stimulated the synthesis of dipalmitoyllecithin in the lung tissue but the substance did not reach the amniotic fluid.

In high risk pregnancies the excretion of urinary estriol is routinely determined. The present study noted a tendency to reduction of this excretion after betamethasone administration which agrees with previous observations (5). This side effect prevents the use of a helpful parameter for assessing placental function.

This study further demonstrates the value of determining the amniotic fluid lecithin/sphingomyelin ratio in high risk pregnancies where premature delivery is considered or imminent. Of the 51 patients 23 had a lecithin/sphingomyelin ratio >2.2 already at the first amniocentesis. In such cases treatment with glucocorticoids with uncertain side effects can be avoided. The results also suggest that glucocorticoid induced acceleration of fetal pulmonary maturation may be reflected in the amniotic fluid as changes in its phospholipid composition.

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THE VALUE OF AMNIOTIC FLUID LECITHIN/SPHINGOMYELIN DETERMINATION IN PREDICTION OF HYALINE MEMBRANE DISEASE

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Abstract 46 samples of amniotic fluid were obtained from 45 women with normal or complicated pregnancy or at parturition. The lecithin/sphingomyelin (L/S) ratio was determined and retrospectively correlated with clinical course of the neonate. Ten neonates developed hyaline membrane disease (HMD). The highest ratio in this group was 1.8. Cesarean section was not associated with HMD if L/S ratio indicated pulmonary maturity (L/S > 1). With the exception of children born by women with toxemia or chronic hypertensive disease, there was no case of HMD among nine infants with birth weights < 2000 g. Developed HMD in about 50% (6/11 cases). The results show that the L/S ratio correlates well to the pulmonary maturity of the neonate. Determination of L/S ratio is therefore of great importance in cases of high risk pregnancy or doubtful gestational age prior to induction or Cesarean section.

inability to meet the demands of postnatal respiratory adjustment by synthesis of sufficient amounts of surface active pulmonary lecithin.

In earlier investigations (15-32) we showed that L/S ratios correlated closely with gestational age in normal and complicated pregnancy and that this could be used to determine gestational age. The purpose of the present study was to determine the reliability of the L/S ratio in predicting the postnatal occurrence of HMD. The incidence of HMD in relation to route of delivery, Apgar score, birth weight and complications of pregnancy such as diabetes, immunization and toxemia were also investigated.

MATERIALS AND METHODS

46 samples of amniotic fluid obtained from 245 pregnancies terminating in the birth of 246 live infants delivered within 3 days of sampling were analysed and L/S ratio and creatinine concentration were determined. 16 of the pregnancies were normal and 84 cases were complicated (Tables I and II). Serial analysis of L/S ratio and creatinine were performed in 6 of the pregnancies. The indication for abdominal amniocentesis was Rh immunization, uncertain gestational age and assessment of possible presence of meconium in high risk pregnancy. In uncomplicated cases, amniotic fluid was collected mostly via amnioscope at delivery. Specimens containing blood or meconium were not included since these substances may interfere with the estimation of L/S-ratio (15). Seven additional samples were obtained from pregnancies resulting in deliveries of dead children.

Assessment of the pregnancies

Toxemia. Diagnosis and classification of toxemia of pregnancy was made according to recommendations of the U.S. Committee on Maternal Welfare (25). To this group

primary distress syndrome or hyaline membrane disease (HMD) is a major cause of neonatal death (3) and antenatal prediction of fetal pulmonary maturity is therefore necessary when in consideration of elective Cesarean section before term is considered. It has been shown by Avery & Mead (4) and others (1) that there is possibly a relationship between a deficiency of surface active material, lecithin, in the lung and later development of hyaline membrane disease in the fetus. In 1969 Nelson (35) suggested that antepartum analysis of amniotic fluid phospholipids might be useful as a method to evaluate the amount of surface active material in the fetal lung. Gluck et al. (22) utilized the lecithin/sphingomyelin concentration ratio (L/S) to predict the amount of surface active material in the fetal lung—the pulmonary maturity. According to Gluck (23) the factors causing HMD seems to be an

Table I Distribution of material in relation to respiratory state of infant

0=no respiratory problem HMD=hyaline membrane disease RD=respiratory distress due to other causes N=246

	0	HMD	RD
Normal	151	6	5
Toxemia	34	1	2
Iso-immunization	32	2	1
Diabetes	11	1	0

In two cases hemorrhage occurred during labor possibly due to placental abruption

belong (a) mild toxemia (b) severe toxemia (c) chronic hypertensive disease

Diabetes mellitus The White classification was used for the patients with diabetes (45)

Iso-immunization Cases were grouped according to Jonasson (29) where group I represents the children with cord hemoglobin ≥ 12 g/100 ml and requiring at most one exchange transfusion group II with cord hemoglobin within the range 8.1–12.0 g/100 ml or requiring 2–3 exchange transfusions and group III with cord hemoglobin 8.0 or lower or requiring more than 3 exchange transfusions

Assessment of the new born

Hyaline membrane disease (HMD) HMD was defined according to Hutchison's criteria (28) difficulty with respiration apparent within 4 hours of delivery persisting for at least 48 hours tachypnea sternal or intercostal recession cyanosis and expiratory grunting and characteristic radiographic changes—diffuse reticulogranular mottling of the lung fields Blood gas studies from umbilical cord demonstrated hypoxemia and acidosis

Table III Respiratory state of infant in relation to different intervals of L/S ratio

0=no respiratory problem HMD=hyaline membrane disease RD=respiratory distress due to other causes

Respiratory states of infant	L/S ratio				
	<1.5	1.5–1.74	1.75–2.0	>2.0	
0	2	3	11	21	
HMD	5	3	2	0	
RD	1	2	2	3	
Incidence of respiratory problem	6/8	5/8	4/15	3/21	
Incidence of HMD	5/8	3/8	2/15	0/15	

Respiratory distress (RD) RD was considered if respiratory difficulties were present but failed to fulfil the criteria for the diagnosis of HMD In most cases other causes could be found for the RD

The L/S ratio in amniotic fluid was determined by modification of Gluck's technique (*) as previously described (15) Amniotic fluid was centrifuged at $1000 \times g$ for 10 min and the supernatant filtered through filter paper To 5 ml of the filtrate 12.5 ml methanol and after shaking 12.5 ml chloroform were added The homogenous phase was allowed to stand for about 30 min at room temperature and then 6.25 ml 0.9 NaCl was added and the mixture shaken (*) After phase separation the lower chloroform phase containing the lipids was recovered and taken dryness under a stream of nitrogen The lipids were dissolved in 100 microliters of chloroform and 10–20 microliters applied to 0.25 mm TLC plates coated with silica H The plates were developed with chloroform/methanol/acetic acid/water 25/15/4/1 (by vol) the spots visualized by charring with H_2SO_4 and L/S ratio determined as previously described (15)

Table II Distribution of material and birthweight (g) of infant and its respiratory state

no respiratory symptoms HMD=hyaline membrane disease RD=respiratory distress due to causes other than HMD N=246 Diagnostic definitions are given in the text

	Weight											
	<1 500			1 500–2 000			2 000–2 500			>2 500		
	0	HMD	RD	0	HMD	RD	0	HMD	RD	0	HMD	RD
Normal		1		1	2	1	5	1	1	145	2	1
Diabetes ABC					1					7		
Diabetes DEF				1			1					
Iso-immunization										70		
Group I				2						8		1
Group II							1					
Group III							3			14		
Toxemia mild						1	2			3		
Toxemia severe	1		1	4			2			5		
Chronic hypertensive disease	1			1			1	1				
Placental abruption			1			1						

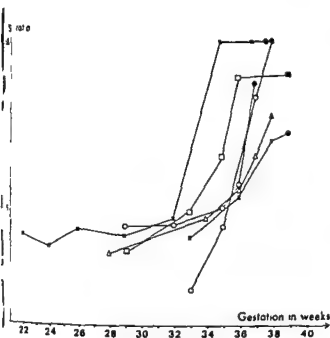


Fig 1 L/S ratio in amniotic fluid versus gestation in weeks serially determined in 6 patients with normal and complicated pregnancy. All the infants healthy. \times normal pregnancy, O iso-immunization, Δ toxemia, \square diabetes. At delivery: \bullet , \blacktriangle , \blacksquare .

RESULTS

Table I lists the distribution of maternal and the incidence of HMD and RD in each group. No significant difference was found between the

Table II shows the classification of maternal and subgroups with regard to birth weight and respiratory status of the infant. With the exception of infants born by women with toxemia or chronic hypertensive disease, where no case of HMD appeared among nine infants, about 50% (6/11) of the infants with birth weight <2000 g in the other developed HMD. Route of delivery and the

respiratory status is presented in Table IV. In this respect there was no appreciable difference between cases of induced and spontaneous labor. Three children delivered by Cesarean section developed HMD, but the gestational age was in two cases incorrectly estimated to be near term (Table VI). Table V shows the incidence of HMD and RD correlated to Apgar scores. Low Apgar scores were found in four cases associated with HMD, i.e. possibly an increased rate in asphyxia.

Table III shows the respiratory status of infants related to L/S ratio. A L/S ratio ≥ 2 suggests pul-

Table IV Number of newborn infants without respiratory problems (0) or developing hyaline membrane disease (HMD), respiratory distress due to other causes (RD) or transient asphyxia postpartum in relation to mode of delivery

	Mode of delivery		
	Spontaneous	Induction with oxytocin	Cesarean section
Respiratory status of infant			
0	139	58	19
HMD	6	1	3
RD	6	1	1
Transient asphyxia	6	4	-

One infant died

Table V Number of cases with hyaline membrane disease (HMD) and respiratory distress due to other causes (RD) related to 5 minute Apgar scores and L/S ratio

	L/S ratio			
	<1.5	$1.5-1.74$	$1.75-2.0$	>2.0
Apgar ≥ 7				
HMD	4	1	1	0
RD	1	1	1	3
Apgar <7				
HMD	1	1	1	0
RD	0	1	1	0

One infant died

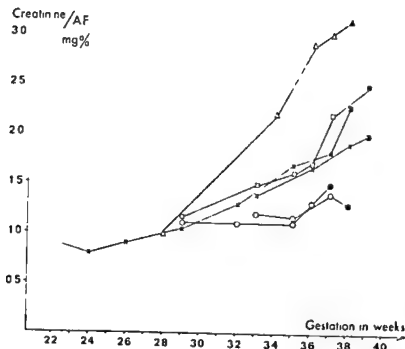


Fig. 2 Creatinine concentration (mg%) in amniotic fluid versus gestation in weeks serially determined in 6 patients with normal and complicated pregnancy. All the infants healthy. × normal pregnancy (o) iso-immunization Δ toxemia (●) diabetes. At delivery ⊙ ● ▲

Table VI Pregnancy and delivery data on patients that delivered infants with subsequent hyaline membrane disease

Case no	Gestational age (weeks)	L/S ratio	Creatinine/AF (mg%)	Maternal complication	Labor	Birth weight (lb)	Sex	Remarks
251	About 35	1.11	1.1	Iso-immunization	Induction	2.000	♂	HMD on X ray. Severe re covered
665	34-38	1.18	1.5	Diabetes (C) and hepatosis	Failed induction and cesarean section	1.8.0	♀	HMD on X ray. Moderate re covered. Probably premature
08	36	1.80	1.5	Intrauterine death x2	Elective cesarean section	2.760	♀	HMD on X ray. Mild recovered
250	33	1.67	1.3	-	Spontaneous	2.030	♂	HMD on X ray. Moderate re covered
552	About 40	1.30	1.5	-	Spontaneous	3.60	♀	HMD on X ray. Moderate re covered
811	32	1.54	1.1	Premature rupture of membranes 24 hours	Spontaneous	1.450	♂	HMD on X ray and at post mortem examination. Died after 7 hours
693	34 or 38	1.42	2.5	Chronic pyelonephritis and hypertension	Elective cesarean section	2.160	♂	HMD on X ray. Probably premature and not small for date. Recovered
178	31	1.82	1.7	-	Spontaneous	1.690	♀	HMD on X ray. Moderate re covered
576	About 30	1.41	1.1	Iso-immunization	Spontaneous	1.540	♂	HMD at postmortem examination. Died after 7 hours
402	33	1.54	1.6	Multiple pregnancy	Spontaneous	1.960	♀	HMD on X ray. Moderate re covered. Twin no II
01	33	2.14	1.5		Spontaneous	2.100	♂	No respiration problems. Twin no I

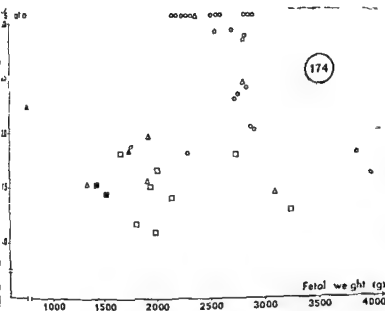


Fig 3 Occurrence of respiratory distress in relation to L/S ratio and birth weight $N=246$

174 infants without respiratory problems ○ no respiratory problems □ HMD recovered ■ HMD died Δ RD recovered (causes other than HMD) ▲ RD died (causes other than HMD)

secondary maturity. In the group with L/S ratio <1.75 10% developed HMD. Fig 3 shows the occurrence of respiratory problems in relation to L/S ratio and birth weight. With a L/S ratio >1.82 no cases of HMD appeared irrespective of birth weight. RD

seemed to be correlated neither to L/S ratio nor birth weight. Serial determination of L/S ratio and creatinine in 6 patients is shown in Figs 1 and 2. It is notable that the toxemia patient had a high creatinine value already in the 34th week while the cor

Table VII Pregnancy and delivery data on patients that delivered infants with respiratory distress (RD) not classified as HMD

Case no	Gestational age (weeks)	L/S ratio	Creatinine/AF (mg%)	Maternal complication	Labor	Birth weight (g)	Sex	Remarks
113	36	5.58	1.9	-	Spontaneous	2400	♀	Transient tachypnea X ray normal
110	37	1.45	1.6	-	Spontaneous	3110	♀	Tachypnea and cyanosis from birth Ductus arteriosus Botalli
171	38	.45	1.4	Iso-immunization	Induction	2850	♂	Exchange transfusion $\times 2$ X ray Pneumonia?
144	35	1.97	1.7	Toxemia	Spontaneous	1960	♂	Intrauterine a. physis X ray normal Recovered after 1 day
137	33	1.57	1.5	-	Spontaneous	1940	♂	Transient tachypnea No X ray
161	About 33	1.54	1.9	Toxemia, placental insufficiency	Cesarean section	1360	♂	Mild transient respiratory problems X ray normal
174	33	1.84	1.4	Placental abruption	Spontaneous	1770	♂	Died 10 min post partum Cerebral hemorrhage at postmortem examination
159	About 7	2.4	-	Placental abruption	Spontaneous	750	♀	Died after 2 days Intra cranial hemorrhage Lungs normal at autopsy

Table VIII Data on patients and infants in cases with oligo or polyhydramnios

Case no	Gestational age (weeks)	L/S ratio	Creatinine/AF (mg%)	Maternal complication	Labor	Birth weight (g)	Sex	Remarks
53	About 30	2.70	1.4	Toxemia placental insufficiency	Induction	970	♀	Intrauterine death 3 weeks after amniocentesis. Dysmaturity. Oligohydramnios.
807	33	1.77	1.6	Toxemia placental insufficiency	Cesarean section	900	♂	No problems of respiration. Delivery 3 days after amniocentesis. Oligohydramnios, slight contamination of meconium.
593	37	1.16	1.5	Duplex	Spontaneous	2070	♂	Died intrauterine. Polyhydramnios. The other twin healthy.
53	41	1.61	-	-	Spontaneous	4380	-	Healthy. 7 l amniotic fluid.
723	39	3.34	-	-	Induction	3500	-	Died in utero 1 day after amniocentesis. Polyhydramnios. 0 anomalies.
550	34	1.40	1.0	-	Spontaneous	1150	-	Died intrauterine. Polyhydramnios. 3.5 l. Multiple anomalies: anencephalic hypoplasia of suprarenal glands.
757	41	1.09	1.9	-	Spontaneous	3160	-	Died intrapartum. Polyhydramnios. 3 l. Multiple anomalies: porencephaly and cranial synostosis.
814	30	1.68	0.9	-	Spontaneous	1350	-	Died intrapartum. Polyhydramnios. 7 l. Multiple anomalies of cor. kidney and micrognathia.
254	41	1.53	-	-	Induction	About 2000	-	Died in utero. Polyhydramnios. Anencephalic.

responding L/S ratio still was low. The spread of L/S ratio in the 35th week was large.

Individual data from ten cases of HMD are collected in Table VI. Case 693 developed HMD; the creatinine value in amniotic fluid was high (Cr/s 1.0).

L/S ratio was low 1.42. The birth weights were 50% ≥ 2000 g. No sex difference was found. The highest L/S ratio in these ten cases was 1.82. The twins (no. 202 and no. 201) had L/S ratios predictive of their individual clinical courses.

Table VII shows individual data from eight cases developing RD. Low L/S ratios were found in case 210 and case 561, but the diagnosis HMD could not be verified. It is of interest that the very premature case 252 had a high L/S ratio and no signs of HMD at autopsy.

Data from two complicated cases of severe placental insufficiency is shown in Table VIII. Seven cases of polyhydramnios are also presented and the majority had multiple anomalies including malformations of the central nervous system. The L/S

ratios were very low in relation to gestational age compared to other pregnancies (32).

DISCUSSION

The clinical value of measuring phospholipids in amniotic fluid as an index of the production of pulmonary surfactant and thereby lung maturity was first studied by Gluck (22) and his conclusion that HMD is unusual when the L/S ratio > 2 was subsequently corroborated by many others (9, 17, 18, 27, 30, 39). The absolute values for the L/S ratio above which HMD is unusual varies between different investigators probably mostly for methodological reasons. With the exception of Schulman (41) most investigators find L/S ratios of 1.5-2 to be values above which HMD is uncommon. This is in good agreement with our results (Table III). Roux et al. (37) on the other hand postulated that the L/S ratio indicates fetal age but not specifically lung

amniotic fluid and changes in amniotic phospholipids following tracheal ligation (7, 8) have however established that there is transport of surfactant from fetal lung to amniotic fluid.

Various ways to determine phospholipid concentration in amniotic fluid have been used (8, 9, 10). The technique employed in this investigation—acetone precipitation omitted (15)—measures the total lecithin fractions and does not alter the L/S ratio compared with other methods (4, 8). Another method of estimating the amount of surfactant is the determination of lecithin concentration (6) but excessive variations in the volume of amniotic fluid may produce misleading results which may be of crucial importance in high risk pregnancies (6, 13). Clements et al (11) have proposed a rapid shake test to be valuable as a screening procedure for determining fetal lung maturity (40, 44).

Not a single case of HMD developed among the low birthweight toxemia infants in contrast to other infants <2000 g (Tables II and VI). The difference might partly be due to the fact that infants in the former group probably were small for dates in some cases and partly that lung maturity seems to be more rapid in cases with toxemia, maternal hypertension (21, 32), small for dates or placental abruption (24) independent of gestational age or birth weight. Toxemia and placental insufficiency might be stress stimuli which raise the fetal plasma corticosteroid levels and cortisol is presumed to be an important physiological inducer of the pathways responsible for the synthesis of surface active material in the lung (31). This would be in agreement with the observations by Murphy (33) who found cortisol levels in umbilical cord blood at delivery to be lower in infants with HMD. This finding was however not confirmed in other investigations (9, 16). It has recently been suggested that the level of total amniotic fluid cortisol may reflect the initiation of fetal lung maturation (14).

Anencephalic fetuses seem to have low amounts of surface active material in the lung (35, Table VII). According to Naeve (34) who found anencephalic neonates with hypoplasia of the adrenal cortex this may be an expression of deficient adrenal function. Reduced adrenal weight in neonates dying from HMD was also reported (34) but not by

HMD is said to occur predominantly in infants delivered by Cesarean section (3, 43) and among infants of diabetic mothers (3). This is however refuted by others (12, 17). Cesarean section is often associated with prematurity but a weight >2500 g does not necessarily exclude the development of HMD (Table VI). The data in this report (Tables I, IV, VI) suggest that delivery by Cesarean section did not increase the incidence of HMD when the fetal lung was mature (L/S >2) prior to operation.

The incidence of HMD associated with L/S ratios <2.0 was 32% (Table III). This is lower than reported by Gluck & Kulovich (21) who found 90% HMD if L/S ratio <2.0 but similar to Spellacy & Buhl (42) and Kalbach (30), 33 and 48% respectively. Differences may be a result of different methodology or the length of time between amniocentesis and delivery.

Low Apgar scores have been suggested to be associated with a higher morbidity from HMD (12). No conclusions can be drawn from our material supporting this because the number of cases is so small but possibly a certain trend in the same direction is present (Table V). According to Gluck the methyl transferase reaction in which palmitoyl myristoyl lecithin—a component of the surface active material in the lung—is synthesized is very sensitive to acidosis (19). This can however hardly be the reason for the proposed association between low Apgar score and HMD (12) as neonates delivered from pregnancies with chronic intrauterine asphyxia tend to develop HMD less frequently compared with normal or other complicated pregnancies (Table II and VIII).

The L/S ratio had a high degree of reliability in predicting fetal lung maturity. A ratio of >2.0 indicated a mature lung and a ratio <1.75 indicated that the risk of development of HMD was very high. Prior to elective Cesarean section or induction it is thus of great importance to determine the L/S ratio if gestational age is uncertain as HMD might appear in spite of a high value of creatinine in amniotic fluid (Table VI, Figs 1 and 2).

In high risk pregnancy the L/S ratio gives useful information to the obstetrician in the decision of the timing of delivery. There is evidence that a damage to the central nervous system can be demonstrated in later life in many children with prolonged exposure to a poor intrauterine environment (46). Thus in certain very high risk pregnancies delivery usually by Cesarean section should be considered.

as soon as the L/S ratio predicts lung maturity (Table VIII) to avoid increased risk of cerebral sequelae or intrauterine death

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A COMPARATIVE STUDY OF PATIENTS WITH CANCER OF THE OVARY WHO HAVE SURVIVED MORE OR LESS THAN 10 YEARS

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Abstract With the purpose of elucidating the characteristics in patients with cancer of the ovary who have survived 10 years compared with patients who have not survived 10 years 161 patients with epithelial cancer and 15 patients with granulosa cell tumor who have survived 10 years group A have been compared with 157 patients with epithelial cancer and 14 patients with granulosa cell tumor who have not survived 10 years group B. The study showed that among epithelial tumors the stage of tumor and the histological picture in the form of 'low potential malignancy' or adenocarcinoma was of decisive importance for 10 years survival. No correlation between formation such as age marital status profession duration of symptoms and the nature of symptoms and 10 years survival could be found in patients with tumor in stages I and II. Neither could the gynaecological examination the nature of the surgical treatment nor radiation therapy be correlated with 10 years survival in stages I and II. Of the 152 patients with epithelial cancer in stages I and II who have survived 10 years have either died from their ovarian disease or have later on developed cancer localized to cervix or corpus uteri. The author points out the risk of not removing both ovaries as well as the uterus and recommends that such patients are followed for many years after the treatment. In 9 patients with granulosa cell tumor neither the medical history the stage of the tumor nor the treatment could be correlated with 10 years survival.

INTRODUCTION

Randall (76) has found in the State of New York that the risk that a woman at some time or other in her life will develop cancer in cervix uteri corpus uteri or in the ovaries is 2.2% 1.5% and 1% respectively. The same author states that the risk that the death of a woman will be caused by the mentioned disease is 0.4% 0.2% and 0.9% respectively. These figures show that ovarian cancer though being the rarest of the three mentioned kinds of tumor causes the highest number of gynaecological cancer deaths.

In spite of the great efforts to improve the prognosis in patients with ovarian cancer by more extensive surgical operations pre and postoperatively high voltage therapy and cytostatic treatment the prognosis at these tumors is still poor and in several of the patients with ovarian cancer a recurrence of the disease has taken place later than 5 years after the primary treatment. Randall (26) The author has in this work tried to elucidate the characteristics of patients with cancer of the ovary who have survived more than 10 years especially with regard to the stage of tumor at the time of operation.

MATERIAL AND METHODS

In Denmark all cases of cancer must be reported to the Danish Cancer Register. This study comprises all women with histologically verified epithelial cancer or with granulosa cell tumor in the ovary who in the period 1943-57 were reported from the city of Copenhagen and Copenhagen and Frederiksberg county. A total of 1165 patients with epithelial ovarian tumors and 29 patients with granulosa cell tumors were registered in the area mentioned between 1943-57.

This study comprises all patients who have survived 10 years 161 patients with epithelial tumor and 15 patients with granulosa cell tumor. These two groups of patients are designated group A. The control group called group B consists of 157 patients with epithelial tumors randomized among the 1004 patients who have not survived 10 years and 14 patients with granulosa cell tumor who neither have survived 10 years.

All the records of the patients have been reviewed by the author and the information below has been procured from these records.

(a) Age marital status profession tumor diagnosed at the prophylactic examination or because of symptoms not related to the abdomen/tumor diagnosed because of symptoms from the abdominal disease previous operation for

Table 1 Distribution of age in patients with primary epithelial carcinoma of the ovary and relationship between stage of the disease and distribution of age

Age	Total				Stage I				Stage II			
	(A) Alive at 10 years		(B) Not alive at 10 years		(A) Alive at 10 years		(B) Not alive at 10 years		(A) Alive at 10 years		(B) Not alive at 10 years	
	No	%	No	%	No	%	No	%	No	%	No	%
Less than 10	0	0	0	0	0	0	0	0	0	0	0	0
10-19 years	1	0.6	2	1.3	1	0.7	1	2.9	0	0	1	2.7
20-29 years	5	3.1	2	1.3	5	3.7	1	2.9	0	0	0	0
30-39 years	24	14.9	7	4.5	19	14.1	3	8.8	4	18	7	5.4
40-49 years	49	30.4	42	26.8	41	30.4	7	0.6	8	36.4	5	13.5
50-59 years	52	33.3	48	30.6	43	31.9	10	29.4	7	31.8	16	43.7
60-69 years	25	15.5	44	28.0	21	15.6	10	29.4	7	9.1	11	29.7
70-79 years	4	2.5	11	7.0	4	3.0	2	5.9	1	4.5	5	5.4
80-89 years	1	0.6	1	0.6	1	0.7	0	0	0	0	0	0
	161	100	157	100	135	100	34	100	22	100	37	100
χ^2	$p < 0.05$				$p > 0.05$				$p > 0.05$			

benign ovary disease (yes/no) duration of symptoms and nature of symptoms

(b) Objective findings at preoperative gynaecological examination clinical stage peroperative rupture of tumor (yes/no)

(c) The nature of the surgical operation radiation treatment (yes/no)

(d) Histological type tumor tissue detected in both ovaries (yes/no) tumor tissue detected in the endometrium (yes/no) and for epithelial tumors also the histological picture ("low potential malignancy /adenocarcinoma)

(e) Possible cause of death secondary tumor detected (yes/no)

Profession When grouping according to profession the division proposed by Svalastoga (9) was used

Clinical stage The division of stage was made according to the division into four stages proposed by the International Federation of Gynaecology and Obstetrics (FIGO) (9)

As the study was made retrospectively it has been difficult to classify the tumor in several of the patients. In cases of doubt between two possible stages the tumor was placed at the stage with the poorest prognosis. The presence of tumor cells was not proved in the ascitic fluid of patients with tumor in stage Ie because such an examination was only rarely carried out

Histology A histological examination of the tumor was made in all patients by 31 different pathologists but the slides were not reviewed. The material is divided into epithelial tumors and granulosa cell tumors. As only 57 of a total of 318 patients with epithelial tumors could be classified as serous mucinous endometrioid or mesonephric as proposed by FIGO (9) a correlation between these types and 10 years survival was not made. Distinction was made between tumors that showed low potential malignancy and adenocarcinoma. The first mentioned group consists of a transition stage between benign and malignant ovary tumors and have been included because these tumors must be treated as a neoplastic disease

which might lead to the death of the patient sometime (17)

Treatment Laparotomy was performed in all patients. 200 patients with epithelial tumor and 17 patients with granulosa cell tumor received radiation treatment. All these patients were irradiated on two dorsal and two ventral areas. 203 received a dosage under 8000 R and 5 received a dosage over 8000 R. 17 patients were additionally given radium therapy. None of the patients received cytostatic treatment.

Collection of information The patients included in the study were given a serial number at random prior to the collection of record information. The purpose was to reduce the influence which knowledge of the survival of the patients might have had on the information recorded.

Statistical analysis To evaluate whether a characteristic appeared more frequently in one of the two study groups than in the other a χ^2 test was used. The 5% limit has been applied as a level of significance. In cases where significant difference was found between group A and B the epithelial tumors were divided according to stage. A re-investigation was then made to see whether a difference could be found between group A and B. Comparison between group A and B could not be made among patients with tumor in stage III-IV due to the small number of patients in group A (Table III).

RESULTS

Epithelial tumors

Characteristics that showed Statistical Difference between group A and B

Distribution on age Table I shows the distribution of age. There is a significant difference between group A and B because there are more young and fewer old patients in the first group. This difference

IV

(B) Not alive
at 10 years

No	%
0	0
0	0
1	1.2
2	2.3
30	34.9
22	25.6
23	26.7
7	8.1
1	1.2
86	100

ence cannot be found after division according to

-46

Symptoms Table II shows those symptoms recorded to be most frequent by Wetterdal (31). The table shows that only symptoms such as poor general condition constipation diarrhoea and vomiting showed a different incidence in the two groups as these symptoms were most frequent in group B. This difference could not be found after division according to stage table II. In stage II pollakisuria was found more frequently in group B than in group A.

Gynaecological examination: preoperative Mobility In 41 patients in group A and in 60 patients in group B the mobility of the tumor was evaluated preoperatively at a gynaecological examination. In group A the tumor was found mobile in 26 patients (63.4%) and fixed in 15 (36.6%). In group B the corresponding figures were 19 (31.7%) and 41 (68.3%). This difference is significant as the mobile

tumors were most frequent in group A. In stage I the tumor was found mobile in 23 patients (67.6%) in group A and in 8 (57.1%) in group B. This difference is not significant. In stage II the number of patients in which the mobility of the tumor was evaluated was too small to make a statistical calculation possible.

Consistency The consistency of the tumor was evaluated in 83 patients in group A and in 90 patients in group B. In group A the tumor was found cystic in 54 patients (65.1%) and solid in 29 (34.9%). In group B the corresponding figures were

40 (44.4%) and 50 (55.6%). The difference is significant as cystic tumors occurred more frequently in group A and solid tumors more frequently in group B. In stage I the tumor was found cystic in 44 patients (65.7%) in group A and in 10 (56.6%) in group B. This difference is not significant. In stage II the number of patients where the consistency of the tumor was evaluated was too small to make a statistical calculation.

The size of the tumor In all patients in group A and B the size of the tumor was evaluated preoperatively. In 89 patients (55.2%) in group A a tumor that measured more than 10 cm in diameter was found and in 51 patients (31.7%) a tumor that measured less than 10 cm in diameter was found. In 21 patients (13.1%) the tumor was not detected preoperatively. In 100 patients (63.8%) in group B a tumor that measured more than 10 cm in diameter was found and in 37 patients (23.7%) a tumor that measured less than 10 cm in diameter was found. In 20 patients (12.5%) the tumor was not detected preoperatively. The difference between the two groups is not significant.

The surface of the tumor In 44 patients in group A and in 83 patients in group B the surface of the tumor was evaluated preoperatively. In group A the surface of the tumor was found to be uneven in 30 patients (68.2%) and even in 14 (31.8%). The figures in group B was 69 (83.1%) and 14 (16.9%). This difference is not significant.

Clinical stage Table III shows group A and B divided according to stage. The table shows that 157 patients (97%) in group A and only 71 (45%) in group B had a tumor in stage I and II. The table also shows that difference in the distribution of stage Ia, Ib and Ic could not be detected between group A and B. Similarly a difference in the distribution of stage IIa and IIb could not be detected.

Surgical treatment Table IV shows that the surgical treatment was different in group A and B. But this difference could neither be found in patients with tumor in stage I nor II (Table IV).

Peroperative rupture In 160 patients in group A and in 135 patients in group B it was stated whether the tumor had ruptured at the operation or not. Rupture occurred in 38 patients in group A (23.8%) and in 65 (48.1%) in group B which is significantly more frequent in group B. In patients with tumor in stage I rupture occurred in 58 (43.3%) in group A and in 20 (62.5%) in group B. This difference is not significant. In patients with tumor in

Table II Distribution of symptoms in patients with primary epithelial carcinoma of the ovary and relation between stage of the disease and symptoms

Symptoms	Total				χ^2 test ($p < 0.05$)	Stage I				χ^2 test ($p < 0.05$)
	(A) Alive at 10 years		(B) Not alive at 10 years			(A) Alive at 10 years		(B) Not alive at 10 years		
	No	%	No	%		No	%	No	%	
Abdominal pain	91	56.5	90	57.3	—	75	55.6	21	61.8	—
Heavy feeling in lower abdomen	31	19.3	29	18.5	—	25	18.5	6	17.6	—
Pollakisuria	36	22.4	45	28.7	—	31	23.0	11	32.4	—
Loss of weight	32	19.9	42	26.8	—	26	19.3	5	14.7	—
Increased girth of the abdomen	62	38.5	58	36.9	—	53	39.3	15	44.1	—
Abdominal tumor	19	11.8	13	8.3	—	15	11.1	3	8.8	—
Metrorrhagia	36	22.4	32	20.4	—	8	7.7	8	23.5	—
Poor general condition	40	24.8	65	41.4	+	36	26.7	9	26.5	—
Constipation	24	14.9	40	25.5	+	20	14.8	5	14.7	—
Diarrhoea	5	3.1	15	9.6	+	4	3.0	1	2.9	—
Vomiting	4	2.5	16	10.2	+	4	3.0	2	5.9	—
Total	161	100	157	100		135	100	34	100	

rupture occurred in 8 patients (36%) in group A and in 10 (27%) in group B. This difference is not significant.

Histology Table V shows that tumors of the type low potential malignancy was more frequent in group A than in group B and that this difference also could be detected in patients with tumor in stage I and II.

Metastases to the other ovary In patients with tumor in stage I both ovaries were histologically examined in 59 patients in group A and in 16 patients in group B. Tumor tissue was detected in both in 18 patients (30.5%) in group A and in 11 (7%) in group B. This difference is significant as

lateral disease was most frequently detected in group B. In patients with tumor in stage II tumor tissue was detected in both ovaries in 9 patients (64%) in group A and in 11 (67%) in group B. This difference is not significant.

Characteristics that did not show any Statistical Difference between Group A and B.

Marital status 99 patients (61.4%) in group A were married and 62 (38.6%) unmarried or previously married. The corresponding figures in group B was 100 (63.8%) and 57 (36.2%). This difference is not significant.

Profession 1 patient (0.8%) in group A belonged to the upper class, 63 (39.2%) to the middle class and 97 (60.0%) to the lower class. 64 patients

(40.8%) in group B belonged to the middle class; 93 (59.2%) to the lower class. This difference is significant.

Reason for admission In 16 patients (9.9%) group A the ovary disease was diagnosed at a prophylactic examination or by an examination of another complaint not related to the abdomen while 145 (90.1%) were examined because of symptoms from their disease. The corresponding figure in group B was 14 (8.9%) and 143 (91.1%). This difference is not significant.

Duration of symptoms 47 patients (30.7%) group A had had symptoms for less than 3 months, 43 (28.1%) for 3-5 months and 63 (41.7%) for more than 5 months. The corresponding figures in group B was 60 (39.2%), 32 (20.9%) and 61 (39.9%). In 12 patients the duration of symptoms could not be estimated. The difference between group A and B is not significant.

Previous operation for benign ovary disease Patients were previously operated for benign ovary disease: 9 belonged to group A and 11 to group B.

Radiation therapy 94 patients (58.4%) in group A and 106 patients (67.5%) in group B had received radiation therapy. This difference is not significant.

Metastases proved in the endometrium In 9 of 64 patients examined in group A and in 5 of 45 examined in group B tumor tissue was detected in the endometrium. This difference is not significant.

II		Stage III-IV				
Alive 10 years	(B) Not alive at 10 years		χ^2 test ($p < 0.05$)	(A) Alive at 10 years	(B) Not alive at 10 years	
	No	%		No	No	%
63.6	18	48.6	—	2	51	59.3
22.7	11	29.7	—	1	12	14.0
18.2	16	43.2	+	1	18	20.9
18.2	9	24.3	—	2	8	32.6
36.4	17	45.9	—	1	26	30.2
13.6	6	16.2	—	1	4	4.7
77.3	9	24.3	—	2	15	17.4
18.2	14	37.8	—	0	45	52.3
13.6	10	27.0	—	1	25	29.1
4.5	3	8.1	—	0	11	12.8
0	4	10.8	—	0	10	11.6
100	37	100		4	86	100

I tumor tissue was proved in 5 out of 44 patients examined with tumor in stage I

Calculation of 10 years survival The study showed that the clinical stage and the histological

Table III Distribution of stage of the disease in patients with primary epithelial carcinoma of the ovary

	(A) Alive at 10 years		(B) Not alive at 10 years	
	No	%	No	%
Stage I	135	83.8	14	21.7
Stage II	22	13.6	37	73.6
Stage III	4	2.6	85	54.1
Stage IV			1	0.6
Total	161	100	157	100
χ^2	$p < 0.05$			
Stage I a	97	68.9	18	5.9
Stage I b	32	23.0	10	29.5
Stage I c	11	8.1	6	17.6
Total	135	100	34	100
χ^2	$p > 0.05$			
Stage II a	4	27.7	9	4.3
Stage II b	18	77.3	8	75.7
Total	22	100	37	100
χ^2	$p > 0.05$			

Ascitic fluid not examined for tumor cells

picture of the tumor in the form of low potential malignancy or adenocarcinoma was of decisive importance to the 10 years survival. As group B is randomized among all patients who have not survived 10 years and therefore must be presumed to constitute a representative section of the patients who have not survived 10 years it is possible to estimate 10 years survival in consideration of clinical stage and histological picture by means of the below formula

$$X = \frac{a \cdot n}{y \cdot z}$$

X = number of patients with survival a clinical stage y and histological picture z

n = total number of patients with survival a

y = the presence of patients with tumor in stage y in patients with survival a

z = the presence of histological picture z in patients with tumor in stage y and survival a

The results of these calculations will appear from Table VI

Cancer in other primary sites 33 patients (20%) in group A and 10 (6%) in group B have after treatment for ovarian cancer been treated for cancer in another site. Cancer of the corpus uteri had been

Table IV Distribution of surgical treatment in patients with primary epithelial carcinoma of the ovary a relationship between treatment and stage of the disease

	Total				Stage I				Stage II			
	(A) Alive at 10 years		(B) Not alive at 10 years		(A) Alive at 10 years		(B) Not alive at 10 years		(A) Alive at 10 years		(B) Not alive at 10 years	
	No	%	No	%	No	%	No	%	No	%	No	%
Explorative laparotomy and diagnostic biopsy	1	0.6	47	29.9	1	0.7	0	0	0	0	5	13.3
One ovary removed	59	36.6	30	19.1	56	41.4	9	26.6	3	13.7	9	74.3
Both ovaries removed	43	26.7	38	24.2	35	26.0	15	44.1	8	36.4	6	16.3
One ovary and uterus removed	9	5.6	6	3.8	8	5.9	2	5.8	1	4.5	3	8.1
Both ovaries and uterus removed	49	30.5	36	23.0	35	26.0	8	23.5	10	45.4	14	37.8
	161	100	157	100	135	100	34	100	72	100	37	100
χ^2	$p < 0.05$				$p > 0.05$				$p > 0.05$			

detected in 10 patients in group A cancer of the cervix uteri in 6 patients and cancer outside the genitalia in 17 patients. In group B 2 patients had developed cancer of the uterine body and 8 patients cancer outside the genitalia after the treatment for cancer ovary. It was not possible to distinguish between metastases and a second primary cancer.

Cause of death 55 patients who have survived 10 years at the conclusion of this study are dead 43 of these patients were originally treated for a tumor in stage I. In 12 (28%) the cause of death was ovarian cancer. 12 of the 55 patients who died were originally treated for a tumor in stage II. In 4 patients the cause of death was cancer of the ovary. 4 patients with tumor in stage III and IV are still alive. In group B all patients are dead. The cause of

death was cancer of the ovary in 20 (59%) patients with tumor in stage I in 27 (73%) with tumor in stage II and in 74 (86%) with tumor in stage III-IV. 36 patients died from other disease.

Granulosa cell tumors

None of the recorded characteristics showed a significant difference between 15 patients with granulosa cell tumor who had survived 10 years in group A and 14 patients who had not survived 10 years group B.

Age The average age in group A was 47.5 years and in group B 51.6 years.

Marital status 9 patients (60%) in group A were

Table V Distribution of histological picture in patients with primary epithelial carcinoma of the ovary and its relation to the stage of the disease

	Total				Stage I				Stage II			
	(A) Alive at 10 years		(B) Not alive at 10 years		(A) Alive at 10 years		(B) Not alive at 10 years		(A) Alive at 10 years		(B) Not alive at 10 years	
	No	%	No	%	No	%	No	%	No	%	No	%
Low potential malignancy	57	38.5	4	2.7	49	39.2	2	6.5	7	35.0	1	2.9
Adenocarcinoma	91	61.5	145	97.3	76	60.8	29	93.5	13	65.0	33	97.1
	148	100	149	100	125	100	31	100	20	100	34	100
χ^2	$p < 0.05$				$p < 0.05$				$p < 0.05$			

II+IV

Years	(B) Not alive at 10 years	
	No	%
47	48	7
17	14	0
17	19	8
1	1	7
14	16	3
86	100	

ned and 6 (40%) unmarried or previously married. The corresponding figures in group B were 8 (53%) and in 6 (36%). This difference is not significant.

Profession 7 patients (47%) in group A belonged to the middle class and 8 (53%) to the lower class. The corresponding figures in group B were 7 (50%) and 7 (50%).

Reason for admission In 3 patients (20%) in group A the ovary disease was detected at a prophylactic examination or at an examination for another complaint not related to the abdomen and 12 (80%) were examined because of symptoms due to their abdominal disease. In group B the corresponding figures were 3 (21%) and 11 (79%).

Duration of symptoms 4 patients (27%) in group

A had had symptoms for less than 3 months (33%) from 3-5 months and 6 (40%) for more than 5 months. In group B the corresponding figures were 6 (43%), 4 (29%) and 4 (28%). Table VII shows that the most frequent symptom was metrorrhagia. None of the symptoms occurred with significantly different frequency in the two groups.

Gynaecological examination 8 patients (52%) in group A were found at the preoperative gynaecological examination to have a tumor with a diameter of more than 10 cm. 5 patients (35%) were found to have a tumor of less than 10 cm in diameter while the tumor was not detected in 2 (13%). The corresponding figures in group B were 9 (64%), 4 (29%) and 1 (7%). This difference is not significant. The consistency, mobility and surface of the tumor was examined in so few patients that a comparison between group A and B was not possible.

Clinical stage Table VIII shows that in group A there is a predominance of patients with tumor in stage I but the difference between group A and B is not significant.

Operative treatment 5 patients (33%) in group A had had a unilateral oophorectomy and 5 (33%) bilateral oophorectomy. 1 (7%) unilateral oophorectomy and hysterectomy and 4 (27%) bilateral oophorectomy and hysterectomy. In group B 1 patient (7%) had had an explorative laparotomy, 4 (28%) unilateral oophorectomy, 5 (37%) bilateral oophorectomy and 4 (28%) bilateral oophorectomy and hysterectomy. There is not a significant difference between group A and B.

Radiation therapy 5 patients (33%) in group A and 7 (50%) in group B received radiation therapy. This difference is not significant.

Metastases to the opposite ovary In group A tumor tissue was detected in both ovaries in 3 out of 6 patients examined and in group B in 1 out of 5 patients examined.

Cancer in other primary sites Cancer was detected in 2 patients after the treatment for ovarian cancer. In 1 patient in group A cancer of the uterine body was diagnosed and in 1 patient in group B cancer of the cervix was diagnosed. It was not possible to distinguish between metastases and a second primary cancer.

Cause of death 3 patients in group A are dead at the conclusion of this study. In 1 patient the cause of death was cancer of the ovary. In 10 patients (71.4%) in group B where all patients are dead the cause of death was cancer of the ovary.

II+IV

Years	(B) Not alive at 10 years	
	No	%
1	1	2
83	98	8
84	100	

Table VI Relationship between 10 years survival stage of the disease and histological picture in patients with primary epithelial carcinoma of the ovary

Stage	No	%	Survival	No	%	Low potential malignancy		Carcinoma	
						No	%	No	%
I	353	30	Alive at 10 years	135	38	53	79	87	79
			Not alive at 10 years	218	62	14	71	04	71
			Total	353	100	67	100	286	100
II	259	22	Alive at 10 years	22	8	8	53	14	6
			Not alive at 10 years	237	92	7	47	730	94
			Total	259	100	15	100	744	100
III+IV	553	48	Alive at 10 years	4	0.7	1	13	3	0.1
			Not alive at 10 years	549	99	7	87	547	99.9
			Total	553	100	8	100	545	100
Total	1 165	100							

DISCUSSION

Epithelial tumors

The purpose of this study was to investigate what differences could be proved between all patients with ovarian cancer who have survived 10 years group A and those who have not survived 10 years group B in a geographically limited area. The most significant difference between the two groups was that 84% of the patients in group A had a tumor in stage I and 16% a tumor in stage II-IV while in group B 22% had a tumor in stage I and 78% a tumor in stage II-IV. A major part of the other differences between group A and B could be ex-

plained as being due to this dissimilar composition of the two groups. Thus a difference in distribution of age, the more frequent appearance of symptoms such as poor general condition, vomiting, constipation and diarrhoea, the more frequent appearance of

solid and fixed tumors and the higher frequency of operative ruptures in group B than in group A could like the actual difference in surgical treatment be explained as being due to the dissimilar representation of patients with tumors in different stages in group A and B. The correlation found between clinical stage and 5 years survival has previously been shown by Mackey & Sellers (18), Munnel (22), Nieminen & Puroila (24) and Pommerehne & Molts (25). The latter also found a marked decrease in 10 years survival from stage I-IV. In the present study no difference in 10 years survival could be found in the distribution of stage Ia, Ib and Ic and stage IIa and IIb among patients with tumor in stage I and II respectively. In agreement with this Aure et al. (2) and Kottmeier (13) could not show a clear decrease in prognosis from stage Ia to Ic. The first mentioned found the same

Table VII Distribution of symptoms in patients with granulosa cell tumors

Symptoms	Alive at 10 y (15 pats)		Not alive at 10 y (14 pats)	
	No	%	No	%
Abdominal pain	6	40.0	6	42.9
Pollakisuria	3	20.0	3	21.4
Loss of weight	1	6.7	1	7.1
Increased girth of the abdomen	1	6.7	1	7.1
Tumor in the abdomen	1	6.7	0	0
Metrorrhagia	9	60.0	10	71.4
Poor general condition	3	20.0	5	35.7
Constipation	0	0	2	14.3

Table VIII Distribution of the stage of disease in patients with granulosa cell tumors

Age	Group A Alive at 10 years		Group B Not alive at 10 years	
	No	%	No	%
I	17	80.0	8	57.2
II	3	20.0	3	21.4
III	0	0	3	21.4
IV	0	0	0	0
Total	15	100	14	100

survival in stage Ib and Ic while the latter found a better prognosis in stage Ic than in Ib. The fact that the ascitic fluid was not examined for tumor cells in the present study renders a direct comparison impossible. Kottmeier (13) found a better prognosis in stage IIa than in IIb which could not be shown in the present study. The histological picture of low potential malignancy or adenocarcinoma has in the present study been of importance for the 10 years survival both in stage I as well as in stage II (Table I). The better prognosis in the first mentioned group is previously shown by Aure et al (2) and Kottmeier (12). Table VI shows the estimated distribution of patients in stage I, II and III-IV in the total population examined and shows that knowledge of the histological picture as well as clinical stage gives a more varied picture of the chance of surviving 10 years than a prognosis merely calculated on the basis of stage.

A poor prognosis of cancer of the ovary in elderly women was previously found by Mackey & Sellers (18) and Pomeroy & Moltz (25). The difference in distribution on age between group A and B is in this work due to the fact that elderly women more frequently had a tumor in stage III-IV than younger women. Women over 60 years of age made up 39% of the patients with a tumor in stage III-IV and only 4% in stage I-II. The poorer prognosis in married women than in unmarried women found by Stone et al (78) could not be shown neither was a correlation between profession and prognosis found. In Stone et al (28) the duration of symptoms could not be related to the prognosis nor was this possible in the present study. Neither was it possible to show a better prognosis in patients in whom the tumor was diagnosed at a prophylactic examination. Only symptoms indicating a widespread tumor (table II)

were more frequent in group B than in group A but a difference in symptoms could not be found after the distribution according to clinical stage. As found by Mackey & Sellers (18), Munnell (21) and Turner et al (30) the most frequent symptoms were pain in the abdomen and abdominal swelling (Table II). The preoperative gynaecological examination forms part of the evaluation of all patients with cancer of the ovary. In the present study the size of tumor and the nature of the surface could not be related to 10 years survival but cystic and mobile tumors were found more frequent in group A than in group B. This difference could however not be found when distributing the patients according to clinical stage.

Aure et al (1), Grogan (6), Mackey & Sellers (18) and Munnell et al (20) found that operative rupture with subsequent risk of intraperitoneal spread did not alter the prognosis. In the present study a correlation between operative rupture and 10 years survival could neither be found in stage I nor II.

Long et al (16), Kent & Mackey (10), Munnell (22) and Pomeroy & Moltz (25) found a better prognosis in patients where both ovaries and uterus were removed at the primary operation. In stage Ia the effect of this extensive surgical operation is however doubtful (Barnes (3), Munnell (23) and Kottmeier (14)). This is particularly the case in patients where the tumor shows a histological picture of low potential malignancy (Kottmeier (14)). In the present study only 25% of the patients with a tumor in stage I and 41% of the patients with a tumor in stage II had both ovaries and uterus removed and the surgical treatment did not have any decisive influence on 10 years survival. A difference in the surgical treatment between group A and B could neither be detected in stage I nor II (table IV). Cancer of the ovary frequently shows metastases to the opposite ovary even though the process is macroscopically unilateral (Munnell (23)) and metastases can be found in the endometrium (Kottmeier (14) and Lynch & Dockerty (17)). In the present study bilateral involvement of the ovary was shown in 29 of 75 patients examined with tumor in stage I and metastases to the endometrium were shown in 5 out of 44 patients examined in this stage. The fact that 18 patients after the treatment for ovarian cancer have developed cancer in the uterus or have had metastases in the uterus indicates the risk of not removing the uterus at the primary operation.

Opinions differ as to the effect of postoperative radiation therapy. Latour & Davis (15), Aure et al (1) and Kottmeier (12) have found a better 5 years survival after radiation therapy to patients with tumor in stage I than if radiation therapy was not given, whereas Munnell & Taylor (19) do not share this view. On the contrary all the mentioned authors recommend radiation therapy to patients with tumor in stage II-IV. In the present study in which 63% of the patients had received radiation therapy, most often a dosage under 8000 R, a correlation with 10 years survival could not be shown. A more close evaluation of the benefit of radiation therapy must take place in the light of a distribution into serous, mucinous, endometrioid and mesonephric tumors, as their sensitivity to radiation seems to vary. The effect of the modern high voltage treatment may also be more effective than the relatively small dosages used in this study.

33 of the 161 patients who have survived 10 years in this study have later developed a new cancer or recurrence, most often in the uterus and in 16 of 55 patients who died more than 10 years after treatment for cancer in the ovary the cause of death was the primary disease. The author must therefore recommend that patients treated for ovarian cancer should be seen for many years after the primary treatment, due to the risk of further cancer or late recurrence.

Granulosa cell tumors

The most pronounced features of the study was that of the characteristics examined showed significant differences in group A and B. Busby et al (4) found a better prognosis in stage I, but in the present study in which 80% in group A and 57% in group B had tumor in stage I, this difference was not significant. The better prognosis found by von Herold (8), Kjellgren (11), Munnell (22) and Sjøstedt & Wahlin (27) in patients with granulosa cell tumors than in patients with epithelial tumors was also found in the present study in which 51% in the first mentioned group and only 13.8% in the last mentioned group have survived 10 years. In the present study the average age was around 50 years, this was also found by Busby et al (4). Sjøstedt & Wahlin (27) found that symptoms such as pain and loss of weight were prognostically bad, but neither these nor the most frequent symptom, metrorrhagia, could in the present study be related to 10 years survival. It is remarkable that this hormone

producing tumor had reached a size of 10 cm in diameter or more in 59% of the patients. This corresponds to the findings in patients with epithelial tumors. Both Munnell (23) and Sjøstedt & Wahlin (27) state that the risk of recurrence is small in patients with the disease localized to only one ovary and they find that unilateral oophorectomy is indicated in young women who have a desire for children. In this study in accordance with this, better prognosis could not be found after bilateral oophorectomy and hysterectomy. Diddle (5) found that granulosa cell tumors are sensitive to radiation, but Haines & Jackson (7) and Sjøstedt & Wahlin (27) could not show a better prognosis after radiation therapy. The last mentioned finding is confirmed in the present study.

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CORRELATION OF MATERNAL PHYSICAL FITNESS DURING PREGNANCY WITH MATERNAL AND FETAL pH AND LACTIC ACID AT DELIVERY

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Abstract The physical fitness of 120 healthy primigravidae was determined two weeks before term using first the standardized three stage submaximal work test and on the following day the voluntarily maximal pulse-conducted work test on a bicycle ergometer. After the first test the level of lactic acid in the capillary circulation of 115 mothers was examined. Immediately after delivery the pH and the level of lactic acid in the maternal artery, umbilical vein and umbilical artery were determined. The level of lactic acid after the work test was negatively correlated with the physical performance of the mother. The level of lactic acid in the mother after the work test and the levels of lactic acid in the umbilical vessels were positively correlated. The mean pH value in physically fit women after delivery was almost significantly higher than in the women of below average performance. The pH level in the umbilical artery was also almost significantly higher in the mothers with above average performance. The physically fit women appear to work more during delivery than the less fit mothers. The level of lactic acid after the delivery was as high as or higher than in mothers with a physical performance below average. The physical fitness of six mothers who delivered an asphyxiated baby was almost significantly lower than the performance of the mothers. Five mothers with an exceptionally high pH after delivery had a significantly higher performance than the other mothers.

During the last twenty years there has been a considerable increase in the understanding of biochemical environment in the mother and fetus during labor. The changes in pH and blood gas levels at the time of delivery have been presented descriptively in several publications (1-9, 10, 11). However, analysis of the reasons for the changes still continues. One point of view is to approach labor in terms of physical performance of the mother.

In work physiology it is well known that physically fit subjects can perform a standardized work

load with a less notable increase in acidic products and with less of decrease in pH than physically unfit subjects (4-16). Also labor is a form of work for the mother. However, there are no earlier investigations on the correlation of the physical fitness of the mother and the levels of pH or lactic acid in the mother and fetus or newborn. Therefore, the topics of investigation in this study were:

- 1) formation of lactic acid in the mother in a standardized work test in late pregnancy
- 2) levels of pH in the mother and newborn after delivery in correlation with the physical performance of the mother
- 3) levels of lactic acid in the mother and in the newborn after delivery in correlation with the physical performance of the mother

SUBJECTS AND METHODS

120 healthy primigravidae aged 20 to 36 years were accepted to take part in the study and gave their consent when informed. The physical fitness of the mothers was determined two weeks before term using two types of work tests on the bicycle ergometer. The first test was a submaximal three stage test with loads of 150, 300 and 450 kpm/min each lasting four minutes in succession (15). The second test the next morning was a pulse-conducted triangular ECG test developed by Arstila (2). The circumstances in the test laboratory and the principles of the tests are described in two earlier papers (6, 7).

Immediately after the first test a blood sample of 0.05 ml was collected from the tip of the warmed middle finger after a deep stab without squeezing ($n=115$).

The deliveries of these mothers were followed and immediately after delivery before the first breath of the newborn the umbilical cord was closed with two clamps and blood samples were taken separately from the umbilical vein (UV) and the umbilical artery (UA). S

Table 1 The results of the work tests \pm SD ($n=120$)

VTW 19%	95.9 \pm 11.8
WL/HR 19%	106.8 \pm 19.2
Mean physical fitness %	101.2 \pm 14.2
Åstrand s index ml/kg min	40.7 \pm 8.5

ously a blood sample from the maternal radial artery (MA) was also drawn. The samples were taken anaerobically in sterile heparinized polyethylene syringes of 5 ml after which the syringes were sealed and placed in ice water.

The pH readings were made on an IL (113 ST) pH blood gas analyzer after calibrating the electrode with Radiometer Copenhagen buffer solutions (7.381 and 6.840 at 38°C). The standard deviation of a single measurement was ± 0.007 pH units when analysis was performed in duplicate.

The blood samples of 200 μ l for the determination of the lactic acid were stabilized with 400 μ l of ice-cold perchloric acid, shaken and stored at +4°C until analyzed. The samples remain unchanged for at least 5 days. For the quantitative determinations the commercially available enzymatic test (Biochemia Boehringer) was used with minor modifications in the technique suggested by the manufacturer. The reliability of the single determination was ± 0.04 mmol/l.

The statistical analysis of the results was performed at the Department of Applied Mathematics of the University of Turku on a Univac 1108 computer.

RESULTS

The results of the work tests are presented in Table I. The mean concentration of the lactic acid after

first work test was 3.20 ± 1.08 (SD) mmol/l, a range of 1.40 to 7.35 mmol/l. The correlations between the results of the work tests and the lactic acid concentration are shown in Table II. All correlations are inversely very significant. The mean pH values after delivery are presented in Table III. In Table IV the pH values are correlated with the results of the pulse conducted triangular ECG test when the mothers have been divided into four subgroups according to the results in the work test. When the physical fitness is presented as WL/HR 19% or as mean physical fitness % the pH value in the MA is almost significantly higher in the groups with a physical fitness of more than 110% than in the groups with a physical fitness of 91–100% (p values < 0.05). When the physical fitness is presented as a percentage of mean physical fitness the pH value in the UA is almost

significantly higher ($p < 0.05$) in the groups with a physical fitness of more than 100% (pH 7.33 ± 0.07 , $n=55$) than in the group of 100% or below (pH 7.30 ± 0.07 , $n=65$).

In six cases the pH value in UV and in UA was lower than two standard deviations from the mean value. The physical performance of those mothers is compared with that of other mothers in Table V and it is shown that the fitness is almost significantly lower. Five mothers had an abnormally high pH diverging more than two standard deviations from the mean pH value. The physical fitness of those mothers is compared in Table VI with that of other mothers and the fitness is significantly higher.

The concentrations of lactic acid after delivery are presented in Table III. There are no significant differences between the levels. Instead all concentrations found after the delivery are very significantly higher than the concentrations after the first work test ($p < 0.001$). The correlations between the lactic acid concentrations are presented in Table VIII. It can be seen that the correlation between the concentrations in maternal capillary blood after the first work test and in maternal arterial blood after the delivery is not significant but there is an almost significant positive correlation between the concentrations in the mother after the work test and in UV and UA after the delivery ($p < 0.05$). The correlations between the lactic acid levels in the maternal and umbilical vessels after delivery are very significant ($p < 0.001$). The concentration of lactic acid in MA after delivery was in 54% of all deliveries higher than in UV and in 51% of deliveries higher than in UA. In Table VIII the parturients have been divided in two groups according to the results in pulse conducted ECG test. When the mean values are compared it is found that the correlation of lactic acid in MA

Table II The correlations between the results of the work tests and the concentration of lactic acid after the work test ($n=115$)

	Correlation coefficient	p
VTW 19%	-0.425	< 0.001
WL/HR 19%	-0.452	< 0.001
Mean physical fitness %	-0.489	< 0.001
Åstrand s index	-0.327	< 0.001

Table III The levels of pH (\pm S D) and lactic acid (\pm S D) in the maternal artery (MA) umbilical vein (UV) and umbilical artery (UA) after delivery

	pH ($n=170$)			Lactic acid mmol/l ($n=170$)		
	Mean	S D	Range	Mean	S D	Range
MA	7.45	0.05	7.33-7.60	4.38	1.57	1.90-9.70
UV	7.37	0.07	7.10-7.49	4.02	1.63	1.95-9.40
UA	7.31	0.07	7.06-7.44	4.07	1.71	1.85-9.25

Table IV The pH values in the maternal artery (MA) umbilical vein (UV) and umbilical artery (UA) after delivery correlated with the physical fitness of the mother ($n=120$)

	$\leq 90\%$	91-100%	101-110%	$>110\%$
$\sqrt{TW} 19\%$	$n=40$	$n=41$	$n=23$	$n=16$
MA	7.44 ± 0.04	7.44 ± 0.05	7.46 ± 0.06	7.45 ± 0.06
UV	7.36 ± 0.07	7.37 ± 0.08	7.37 ± 0.08	7.37 ± 0.06
UA	7.30 ± 0.07	7.32 ± 0.08	7.32 ± 0.08	7.32 ± 0.06
$WL/HR 19\%$	$n=19$	$n=35$	$n=22$	$n=44$
MA	7.44 ± 0.04	7.44 ± 0.04	7.44 ± 0.05	7.46 ± 0.06
UV	7.36 ± 0.07	7.35 ± 0.08	7.38 ± 0.07	7.38 ± 0.07
UA	7.31 ± 0.07	7.30 ± 0.08	7.31 ± 0.07	7.33 ± 0.07
Mean physical fitness %	$n=24$	$n=41$	$n=28$	$n=27$
MA	7.44 ± 0.04	7.44 ± 0.04	7.45 ± 0.05	7.47 ± 0.06
UV	7.36 ± 0.07	7.36 ± 0.08	7.37 ± 0.08	7.38 ± 0.06
UA	7.31 ± 0.07	7.30 ± 0.07	7.32 ± 0.08	7.33 ± 0.06

after the delivery is very significantly higher in the mothers whose $\sqrt{TW} 19\%$ values were above average. In the same group the concentration of lactic acid in UV was almost significantly higher (<0.05) than in the group with $\sqrt{TW} 19\%$ below average. Other differences are not statistically significant.

Table V Comparison between the physical fitness (\pm S D) of mothers with an abnormally low umbilical cord pH and the fitness of mothers with a normal umbilical cord pH post partum (Cochran's test)

	Mothers with an abnormally low umbilical cord pH ($n=6$)	Mothers with a normal umbilical cord pH ($n=114$)	P
$\sqrt{TW} 19\%$	93.0 ± 11.9	96.0 ± 11.8	<0.05
$WL/HR 19\%$	94.5 ± 8.8	107.1 ± 19.6	<0.01
Mean physical fitness %	93.7 ± 8.9	101.6 ± 14.4	<0.05

DISCUSSION

A decrease of pH in the mother and fetus during the end of the first stage and in the course of the second stage of labor is well documented (1-9, 10, 11). In the mother this has been attributed mainly to the accumulation of lactic acid as a result of uterine activity and the voluntary muscular efforts of the mother during labor (3, 14). In the fetus the decrease of pH is generally of both a metabolic and respiratory type. A notable lowering of pH in the fetus means a threat to fetal well being.

The mean pH values found in this study are comparable with other studies (9, 10, 11). When the pH values are correlated with physical fitness, a certain relationship is noticed. The asphyxiated babies were delivered by mothers whose physical performance was lower than average. The mothers with an exceptionally high pH after delivery, in other words mothers with probable respiratory alkalosis, tended to have better physical performance than average. Also the normal pH values are in some distinct positively correlated with the physical fitness of the mothers.

Table VI Comparison between physical fitness ($\pm S D$) of mothers with abnormally high arterial pH and that of mothers with normal pH post partum (Cochran's test)

	Mothers with abnormally high arterial pH (n=5)	Mothers with normal arterial pH (n=115)	p
\sqrt{TW} 19%	106.4 \pm 8.4	95.5 \pm 11.5	<0.01
WL/HR 19	122.6 \pm 14.0	105.8 \pm 19.3	<0.05
Mean physical fitness %	114.4 \pm 8.8	100.6 \pm 14.2	<0.01

The question remains why the pH values of physically fit mothers and those of their babies are higher. There are two possible explanations for the maternal values. First, the oxygenative system of the physically fit women could be more capable of eliminating CO_2 compared with the elimination rate in unfit mothers. Second, the physically fit mothers could be more capable of producing energy by means of the oxygenative system requiring less anaerobically produced energy. The increase of acid metabolites, which is found to occur during the second stage of the labor (3-9, 14), would then be less.

The pH changes in the fetus in normal labors are for the greatest part generated by maternal influences (3-9, 14) but at the end of the second stage, probably due to disturbances in utero-placental circulation, an inadequate oxygen supply to the fetus may occur (9) and the amount of H^+ ions in the fetus increases. Thus the reasons for the higher pH of the newborn of fit mothers could be either the lower concentration of acid metabolites in the mother to transfer to the fetus or a better circulation in the uterus and placenta to produce the better

Table VII The correlations between the concentrations of lactic acid in maternal arterialised capillary sample after the work test (MC work) in maternal radial artery after the delivery (MA) in umbilical vein (UV) and in umbilical artery (UA) (n=115)

	MA	UV	UA
MC work	0.123	0.203	0.215
MA	—	0.638*	0.561*
UV	—	—	0.954*

p<0.05 * p<0.001

Table VIII The comparison of lactic acid concentrations ($\pm S D$) in the groups of physical performance below and over normal (n=100)

	$\leq 100\%$	$> 100\%$	p
\sqrt{TW} 19%	n=76	n=39	
MA	4.04 \pm 1.34	5.05 \pm 1.79	<0.001
UV	3.79 \pm 1.51	4.49 \pm 1.77	<0.05
UA	3.85 \pm 1.63	4.51 \pm 1.80	n.s.
WL/HR 19%	n=50	n=65	
MA	4.45 \pm 1.58	4.33 \pm 1.58	n.s.
UV	4.17 \pm 1.75	3.91 \pm 1.54	n.s.
UA	4.23 \pm 1.80	3.95 \pm 1.67	n.s.
Mean physical fitness %	n=61	n=54	
	4.23 \pm 1.51	4.55 \pm 1.65	n.s.
	3.94 \pm 1.69	4.12 \pm 1.58	n.s.
	3.96 \pm 1.70	4.19 \pm 1.66	n.s.

n.s. = not significant

elimination of CO_2 from the fetal blood. Support for the latter idea is given by the finding that placenta of fit women are significantly heavier than those of less fit women (Erkkola, unpublished observation).

The lactic acid level in the three stage work test of this study is negatively correlated with the physical fitness, which was to be expected in light of other studies (4, 16). When the lactic acid levels after the work test and after delivery are compared, it can be seen that in any case labor has been harder work than 3600 kpm in 12 minutes. The lactic acid levels after delivery found in this study are well comparable with the results of other studies (9), especially since all parturients were primigravidae. (3) The mean concentration of lactic acid in the mother 4.38 mmol/l after labor is not very high. In some individual cases, however, concentrations of 7 to 9 mmol/l were found, indicating that during the last minutes of labor the mother had an intensive anaerobic working period.

If we compare the concentrations of lactic acid in the mother and in the umbilical vessels, it becomes clear that in most cases the main source of the fetal lactic acid is the mother. The lactic acid passes the placenta freely in monkeys (8) and it is presumed that a free crossing occurs in man as well (3, 9). However, it is also clear that in some cases due to hypoxia during the last minutes of labor the fetus produces its energy through anaerobic metabolism, resulting in high levels of lactic acid in spite of low levels in the mother.

When the lactic acid levels after the work test and after labor are correlated, some interesting findings are made. There is no significant correlation between the maternal concentrations. Logically it could be presumed that the production of energy is of the same relative magnitude in the work during labor as in the work test. When the correlation between the maternal lactic acid concentration after the work test and the concentrations in the umbilical vessels are calculated, it is found that they are almost significant. This finding is difficult to explain since the correlation between the maternal concentrations after test and labor was not significant in this material. However, it is known that during exercise the greater part of cardiac output circulates through the working muscles. Thus, when pregnant women are exercising, the circulation in the uterus diminishes and after the exercise the circulation overflows in the uterus occurs to compensate a possible insufficiency in the fetal oxygenation (5, 12). The higher concentration of lactic acid in the mother after exercise may indicate a lower oxygenative capacity. This may cause disturbances in the utero-placental flow during labor, resulting in an elevated anaerobic metabolism in the fetus, the products of which can not be eliminated by the mother after the delivery.

When the levels of lactic acid in MA, UV and UA are correlated with physical fitness, an interesting finding is made. The mean concentration of lactic acid in the mothers with \sqrt{TW} 19% over 100% is significantly higher than that in mothers with a \sqrt{TW} 19% below 100%. Since the lactic acid level in the standardized work was lower in physically fit women than in unfit mothers, the only explanation is that the mothers with higher \sqrt{TW} 19% have been working more during delivery. On the other hand, there are no statistically significant differences between the levels of lactic acid when the physical fitness is presented as a percentage of $W/L/HR$ 19 or as a percentage of mean physical fitness. These results show that the physically fit mothers do not produce less lactic acid than the less fit mothers during labor. Therefore, it could be presumed that they are working more during the second stage and especially during the period of expulsion of the baby. This finding of similar or higher levels of lactic acid with fit mothers differs from the results of Stembera et al. (13) who found that the lactate production was less in women who had a gymnastic program during the pregnancy when

compared with sedentary mothers. They did not, however, take the level after delivery but measured the rise of concentration every three hours during labor. Also the difference began to be noticed after labor of 10 or more hours duration, whereas in this study the mean duration of labor was about 7-8 hours (Erkkola, unpublished observations).

Since the pH levels of fit women were higher than the pH levels of less fit women, it logically follows that the respiration and oxygenative capacity of fit mothers compensate the higher or equal levels of lactic acid. Five women with exceptionally high pH values and simultaneously with high physical capacity give support to this theory.

The following conclusions from the results of this study can be made:

- 1) The more the mother produces lactic acid during the standardized work test, the higher are the concentrations of lactic acid in the umbilical vessels after the delivery.
- 2) The pH in fit mothers is higher than the pH in less physically fit mothers after the delivery. This is reflected to some extent in the fetal pH.
- 3) The fit mothers perform as much anaerobic work during labor as the less fit mothers.
- 4) The high levels of lactic acid and thus the metabolic acidosis were compensated by the high oxygenative capacity of the physically fit women.
- 5) The mothers with low physical performance are more liable to have asphyxiated babies than the physically fit mothers.

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CHANGES IN CARDIAC FUNCTION DURING AND AFTER PREGNANCY EXPRESSED BY SYSTOLIC TIME INTERVALS

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Abstract Hemodynamic changes during the first and third trimester in pregnancy and in the first week of puerperium were evaluated by non invasive measurements of Systolic Time Intervals (STI) in supine and left lateral position. The Pre-ejection Period (PEP) was found to shorten significantly in pregnancy and the puerperium due to the increased blood volume. The electro-mechanical systole (QS) and left ventricular ejection time (LVET) were shortened too while PEP/LVET ratio was increased in the third trimester due to the mechanical compression of the gravid uterus on the inferior vena cava. A lengthening of QS and LVET and a decreased PEP and P/L ratio were demonstrated in the third trimester in lateral position when the pressure of the enlarged uterus was eliminated. Heart rate (HR) increased in late pregnancy as well as after delivery while arterial blood pressure (BP) only underwent minor changes. Employment of the STI seems to provide more useful information about the changes in cardiac function during gestation than HR and BP does. The measurements of STI can be repeated without any risk or inconvenience to the patients.

INTRODUCTION

Physiological changes develop in many organ systems during the course of gestation and parturition (7). The changes in the maternal cardiovascular system are related to hemodynamic, volumetric, hormonal and nutritional factors (22).

Cardiac output measured by either direct Fick principle (6) or by dye dilution technique (21) has shown an increase from the first trimester with a maximum between the 24th and 32nd week of gestation. In late pregnancy cardiac output remains high unless the pregnant woman assumes a supine position

(14) when the enlarged uterus compresses the inferior vena cava (9) and abdominal aorta (2) which can produce the supine hypotensive syndrome (13, 18).

Evaluation of myocardial function by Systolic Time Intervals (STI) is based on the classic studies by Wiggers (27) and Katz and Feil (12). It is now commonly agreed that STI are useful as non invasive indices of cardiac function (4, 5, 25, 26). The pre-ejection period (PEP) is closely related to the isovolumic contraction time (15) and left ventricular ejection time (LVET) vary directly with stroke volume (24). The ratio between PEP and LVET (PEP/LVET ratio) has been shown to be a semiquantitative index of myocardial function (24).

The purpose of this study was by non invasive means (STI, heart rate, arterial blood pressure) to record the changes in cardiac function during pregnancy (the first and third trimester) and the first week of the puerperium together with the response to change in position (supine/left lateral).

MATERIAL AND METHODS

The STI of the left ventricular systole were measured from simultaneous recordings of electrocardiogram (ECG), phonocardiogram (phono) and right carotid arterial pulse tracing (Fig. 1). The recordings were obtained on an Elema Mingograph 34[®] recorder at a paper speed of 50 mm/sec. The electrocardiographic lead II was employed. A phonomicrophone (EMT 21B amplifier EMT 28B) was placed in the third left intercostal space lateral to the sternum for recording the second heart sound (S₂). The

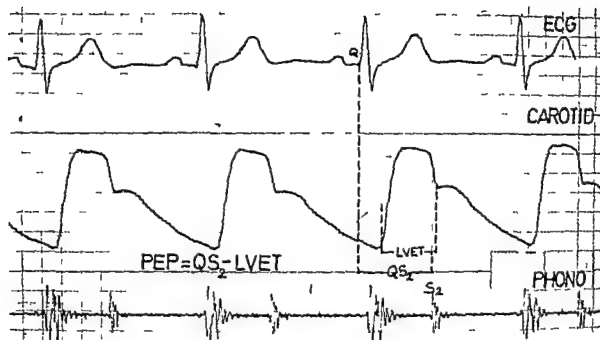


Fig 1 Original tracing showing measurements of QS_2 (total electromechanical systole) LVET (left ventricular ejection time) and PEP (pre ejection period) from elec-

trocardiogram (ECG) carotid pulse curve (carotid) and phonocardiogram (phono)

carotid pulse curve was recorded with a pressure sensitive pulse receptor (EMT 439A amplifier EMT 29) placed over the right carotid artery

The following STI were measured (see Fig 1) 1) Total electro mechanical systole (QS_2) from the onset of the Q wave of the ECG to the initial high frequency vibration of the second heart sound (S_2) 2) Left ventricular ejection time (LVET) from the upstroke of the carotid pulse tracing to the diastolic notch and 3) Pre ejection period (PEP) derived by subtracting LVET from QS_2 . All intervals were calculated as the mean of ten consecutive beats without correction for HR. The ratio between PEP and LVET (PEP/LVET ratio) was calculated from the values of PEP and LVET.

Heart rate (HR) was estimated from the R-R interval and arterial blood pressure (BP) was measured by the Riva Rocci method.

Thirteen non pregnant women aged 21–32 years (mean 26 years) were used as control group.

Thirtyeight women aged 19–47 years (mean 28 years) in the 8th–12th week of gestation (mean 10 weeks) were studied when admitted for induced abortion (first trimester).

Eleven women aged 27–39 years (mean 28 years) undergoing elective Caesarian section were studied the day before surgery (third trimester) and again eight days later (puerperium).

None of the operations were performed on account of maternal disease.

The protocol consisted of a 15 minutes stabilization period after which STI, HR and BP were obtained in supine and then in left lateral position. Three consecutive measurements were obtained in each position with an interval of 5 minutes and then averaged.

Table 1 STI, BP and HR obtained in supine position in non pregnant women during pregnancy and in puerperium (mean values \pm S.D.)

STI=systolic time intervals QS_2 =total electromechanical systole LVET=left ventricular ejection time PEP=pre-ejection period BP=arterial blood pressure HR=heart rate

	QS_2 (msec)	LVET (msec)	PEP (msec)	PEP/LVET (msec/msec)	BP (mmHg)	HR (beats/min)
Non pregnant	416 \pm 28	300 \pm 26	116 \pm 16	0.39 \pm 0.07		68 \pm 13
1st trimester	392 \pm 26	301 \pm 20	91 \pm 14	0.31 \pm 0.05	117/77 \pm 10/7	73 \pm 17
3rd trimester	356 \pm 29	267 \pm 18	89 \pm 20	0.34 \pm 0.08	124/81 \pm 15/11	86 \pm 8
Puerperium	380 \pm 25	298 \pm 17	82 \pm 18	0.28 \pm 0.06	117/78 \pm 14/11	79 \pm 8

Table II Mean values (\pm SD) of STI, BP and HR measured in the left lateral position during pregnancy and in puerperium

Abbreviations as Table I

	QS ₂ (msec)	LVET (msec)	PEP (msec)	PEP/LVET (msec/msec)	BP (mmHg)	HR (beats/min)
1st trimester	397 \pm 74	305 \pm 18	92 \pm 15	0.31 \pm 0.05	101/59 \pm 12/10	73 \pm 10
2nd trimester	372 \pm 23	290 \pm 14	82 \pm 17	0.29 \pm 0.06	118/83 \pm 15/13	81 \pm 8
Puerperium	387 \pm 31	300 \pm 72	85 \pm 21	0.29 \pm 0.07	113/77 \pm 18/12	77 \pm 9

Statistical analysis of the data was based on Wilcoxon tests for paired and unpaired data and Student's *t* test for unpaired data.

RESULTS

The supine position (Table I)

When compared with the non pregnant (control group) QS₂ and PEP were significantly shorter in the other periods ($p < 0.01$). PEP/LVET ratio showed a decrease in the first trimester and in the puerperium ($p < 0.01$) while no difference was observed in the third trimester because of the concomitant shortening of LVET ($p < 0.01$). HR did only increase in the third trimester ($p < 0.01$) and in the puerperium ($p < 0.05$).

Before Caesarian section QS₂ and LVET were shorter ($p < 0.02$, $p < 0.01$) and PEP/LVET ratio increased ($p < 0.02$) when compared to the values obtained eight days later. HR was higher before delivery ($p < 0.02$) while no difference in BP was observed.

By comparing the third trimester with the first trimester a shorter QS₂ and LVET ($p < 0.01$) as associated with an increased HR ($p < 0.01$) was found in the third trimester while no differences in BP were found.

The values obtained in the puerperium did not differ from the first trimester values apart from the higher HR ($p < 0.02$).

The left lateral position (Table II)

In the third trimester a shorter QS₂ and LVET ($p < 0.01$) and a higher HR and BP ($p < 0.01$) were found when compared with first trimester.

No significant differences were found between the values obtained before and after Caesarian section. Nor did the STI in the puerperium differ from the STI in the first trimester besides HR and BP were slightly higher in the former group ($p < 0.02$, $p < 0.05$).

The effects of changes in position (Table III)

A significant lengthening in QS₂ and LVET ($p < 0.01$) and decrease in PEP and PEP/LVET ratio ($p < 0.01$, $p < 0.02$) were observed in the third trimester when the lateral position was assumed. Any changes in HR and BP were not significant.

In contrast the parameters obtained in the puerperium and first trimester showed no significant differences with change in position apart from a reduction in BP ($p < 0.01$) in the first trimester.

DISCUSSION

The increase in blood volume and cardiac output begins in the late first trimester (10, 11, 16, 22). Blood volume reaches maximum in the third trimester and cardiac output peaks between 24th and

Table III Significant levels in STI, BP and HR by changing position from supine to left lateral

Abbreviations as Table I

	QS ₂	LVET	PEP	PEP/LVET	BP	HR
1st trimester	NS	NS	NS	NS	$p < 0.01$	NS
3rd trimester	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.02$	NS	NS
Puerperium	NS	NS	NS	NS	NS	NS

NS = not significant

32nd week to decline later due to abdominal vascular compression (6 13 21). The rise in cardiac output is brought about by an increased HR and an increased stroke volume (11). Non pregnant levels in blood volume and cardiac output has been reached in the 2nd week of puerperium (16 22).

These hemodynamic changes are in part induced by the increased estrogen and thyroxine production which influence blood volume as well as the strength of myocardial contraction (7 20).

The hemodynamic changes during pregnancy and the first week of puerperium were reflected in all parameters employed in this study.

A shorter PEP and a decreased PEP/LVET ratio in the first trimester were found due to the increased blood volume and increased venous return leading to an increased preload. The increase in preload results in a shortening of PEP and a decrease in PEP/LVET ratio (24). An increased level of circulating plasma catecholamines due to anxiety and the increase in thyroxine production will further abbreviate PEP (1 8).

Changing to left lateral position in the first trimester does not affect STI or HR but results in a reduction in arterial blood pressure (i.e. a decrease in afterload). A fall in systolic and diastolic arterial blood pressure in the left lateral position has been described in early pregnancy when recorded by brachial sphygmomanometry (19) and this decrease in afterload induce only minor changes in STI.

The compression of the inferior caval vein and the abdominal aorta in the third trimester will lead to a reduction in preload in the supine position and concomitant changes in STI. This is further demonstrated when the left lateral position is adopted and the compression thus eliminated: the enhanced ventricular filling which follows the changes in position is clearly reflected in the lengthening in LVET, abbreviation of PEP and decrease in PEP/LVET ratio (18 24). These signs of improved cardiac performance are also found in the same women 8 days after delivery independent of change of position.

However, when the values in the first week of puerperium were compared to those of the control group, PEP and PEP/LVET ratio were still shorter postpartum which is in agreement with the fact that blood volume in the first week of the puerperium still has not returned to non pregnant levels (16 22). A further evidence for this explanation was demon-

strated by comparing the STI from the first trimester with the STI obtained in the puerperium. We found no significant differences in any STI between the two groups in either position due to an increased blood volume in both groups.

Burg et al (3) have demonstrated a shortening of PEP in the second trimester due to an enhanced myocardial contractility secondary to the increased diastolic filling. In the third trimester they found besides a shortening in QS₂ and LVET a lengthening in PEP and PEP/LVET ratio in the supine position because of the impaired venous return while PEP and PEP/LVET ratio in the left lateral position did not differ from predicted values.

Rubler et al (17) measured STI on 16 women in supine and sitting positions during pregnancy and six weeks postpartum. They found a shorter PEP at the time of maximum expansion of blood volume compared to PEP in the postpartum period which is in agreement with our findings too.

In conclusion, the non invasively measured STI provide us with more sensitive information about the cardio vascular changes during and after pregnancy than the conventional measurements of heart rate and arterial blood pressure. The employment of STI is a useful aid in assessing cardiovascular irregularities in the ante partum and post partum periods. The measurement can be repeated frequently without any risk or inconvenience to the patients.

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A COMPARATIVE STUDY OF UTERINE ACTIVITY IN LABOUR INDUCED WITH PROSTAGLANDIN I₂ OR OXYTOCIN AND IN SPONTANEOUS LABOUR

1 Pattern of the Uterine Contractions

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Abstract The uterine contraction patterns and the changes in fetal heart rate (FHR) were studied in cardiotocographic recordings from 6 women in oxytocin-induced labour, 26 women in PGF_{2α} induced labour and 4 women during the later part of spontaneous labour. The contraction patterns and their effect on the FHR did not differ between the three groups. During the course of labour an increasing steepness of the upward slope of the contraction wave with increasing intensity of the contraction was found. High frequency of atypical contraction patterns suggesting some degree of uterine incoordination was found during the active phase of labour in 10 patients, 8 of whom were primiparae. This incoordination could not be related to the effect of induction with either drug. Incoordinated contractions were associated with longer duration of labour and a tendency to more pronounced acidosis in the infant at birth, although mean values still fell in the normal range. Ominous FHR patterns were only seen in 2 cases of uterine hyperactivity during induction of labour.

Intra-uterine pressure recordings have been used to great extent for research work and also in routine obstetrics. In spite of this fact relatively little attention has been paid to the shape and pattern of the uterine contractions as found on pressure recordings in connection with spontaneous and induced labour. It seems to be more or less generally accepted that in normal labour most contractions have a regular form like a Gaussian curve.

Baumgarten (3) studied the form of the uterine contractions during normal labour. He classified their shapes and categorized them into three different groups based on the relation between the length of the upward and downward slopes of the

contraction waves (Fig. 2). He observed that with advancing labour higher frequencies of contractions with a steep rise and slow decline were seen and concluded that this type was more effective than other forms in achieving a rapid progress of labour.

In the course of his studies of the uterine activity during labour Caldeyro-Barcia (1, 9) concluded that atypical patterns in the intra-amniotic pressure curve could be the result of incoordinated myometrial activity. In this type of labour a new contraction is often superimposed upon the down-slope of the preceding one or starts very close to it. For this reason the normal decline of the pressure curve to the basal tone level between contractions fails to appear either for longer or shorter periods. When incoordination is pronounced it causes slow progress of labour.

From clinical trials with prostaglandins used for labour induction a high incidence of incoordinate labour patterns have repeatedly been reported (25, 30, 34). There are however also reports which state that aberrant labour patterns often occur during spontaneous labour and oxytocin infusions (1, 11, 13, 35, 39, 40). Few reports seem to have dealt with the question of the incidence of these incoordinations in induced or in spontaneous labour. Because the uteroplacental blood flow and hence the fetal well-being are highly dependent on uterine function during labour it was thought worth while to compare the pattern of uterine contractility in prostaglandin induced labours with that found in oxytocin-induced or spontaneous labour.

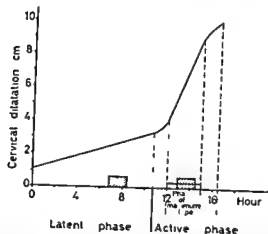


Fig 1 The periods of detailed study of the cardiotocographic recordings indicated (hatched areas) in a partogram. After Friedman (14)

MATERIAL AND METHODS

The material comprised 76 women studied during labour. In 26 cases labour was induced with prostaglandin $F_{2\alpha}$ (Group P) and in 26 cases with oxytocin (Group O). Both drugs were given by intravenous infusion at a rate of 3–15 $\mu\text{g}/\text{min}$ for $\text{PGF}_{2\alpha}$ and of 2–16 mU/min for oxytocin. Twenty four women in spontaneous labour served as controls (Group N). They were not given any oxytocic drugs. Conduction anesthesia was not used. Injections of pethidine and diazepam or inhalations of 50% nitrous oxide/oxygen were used for pain relief. Parity and other pertinent clinical data for the patients are given in Table I. All the infants were liveborn and healthy and the presentations were normal occiput–anterior vertex. Bishop score (5) was always assessed prior to induction.

In all cases the intra uterine pressure and the fetal heart rate were monitored with cardiotocographic technique. Fetal heart rate was recorded on cardiotocographs from Hewlett Packard and Corometrics were used. Open end polyethylene catheters filled with 0.9% saline were used for the pressure recordings. In the induction cases the catheters were introduced before the

start of the infusion and placed extra amniotically where in most cases could be done without rupturing the membranes. The validity of intra uterine extra-ovular pressure measurements has been discussed by Csapo (17) and Hendricks (17). We were able to obtain good recordings in most cases by repeated flushing of the catheter with 0.9% saline. In cases of spontaneous labour recordings were started not later than at cervical dilatation of 4 cm. At this stage the membranes were artificially ruptured in all patients if spontaneous rupture had not already occurred.

The fetal heart rate (FHR) was recorded with ultrasound monitors or by phonocardiography before the membranes were ruptured and thereafter with silver/silverchloride electrodes of the clip or spiral type attached directly to the fetal scalp. The clinical course of labour was recorded on partograms as proposed by Friedman (14).

In the pressure recordings 20 consecutive contractions were studied during each of two different periods of labour. The first period corresponds to the late latent phase when regular labour was established and the second to the phase of maximum slope in the partogram curve (Fig 1). We were not able to study the latent phase in the patients with spontaneous labour because most had not come to the delivery ward at this stage of labour. In 12 cases of induction of labour (6 from the O group and 6 from the P group) it was not possible to obtain satisfactory intrauterine pressure recordings from the early part of the first stage. This was due either to technical difficulties with the catheter or the absence of a well defined latent period due to rapid cervical dilatation. A pressure recording from the active phase in one multipara in the O group had to be discarded for technical reasons.

Pressure recordings covering both periods described above were studied and the contractions classified according to the method described by Baumgarten (18). Atypical contraction patterns were noted separately. Atypical contractions were recorded. Contractions with a second wave immediately superimposed upon the first one and all pairs of twos of contractions in which the basal tone level was not reached between the contractions because the interval between was too short. In 16 random

Table I Pertinent clinical data for the three groups of patients

	Spontaneous N		Oxytocin O		PGF _{2α} P	
	PP	MP	PP	MP	PP	MP
N	12	12	12	14	12	14
Weeks of pregnancy						
Means	40.9	40.6	41.9	41.3	40.6	39.1
Range		38–42		40–43		36–43
Pelvic score (means)			7.0	6.8	4.8	4.5
Birth weight grams (means)	3.657	3.663	3.587	3.756	3.385	3.5.8
Total length of 1st stage (hours)						
(means \pm 1 S.D.)	11.4 \pm 5.1	7.1 \pm 3.8	7.2 \pm 5.5	3.4 \pm 2.2	6.4 \pm 3.4	4.1 \pm 1.1
Total length of 2nd stage (minutes)						
(means \pm 1 S.D.)	60 \pm 39	15 \pm 11	43 \pm 0	11 \pm 16	37 \pm 31	7 \pm 5

	Type I	Type II	Type III	
Latent phase	O 36 P 116	O 602 P 513	O 314 P 271	O 46 P 106
Active phase	O 33 P 10	O 376 P 429	O 429 P 442	O 116 P 37

Fig 2 Relative frequencies in per cent of the different types of contractions found during the observation periods in the three groups of patients

selected patients 8 from each of the Groups O and P the duration and the amplitude of the contractions was studied more closely. The duration was measured to the nearest 0.1 minute at the level of the basal tonus and the intensity to the nearest 5 mmHg.

Values for pH, pCO_2 and BD_{ext} were assessed in blood from the umbilical artery in 35 newborn babies.

After the membranes were ruptured the fetal heart rate changes during the rest of the first stage were studied. The changes were classified according to the terminology of Hon (19).

RESULTS

The shape of the uterine contraction waves

In order to get a general idea of the overall distribution of the different types of contractions in the groups all contractions studied during each part of labour were pooled for the patients in each group. The relative frequencies of the different types of contractions subsequently found in the three groups of patients are shown in Fig 2. Contractions of type II and III were found in approximately the same proportions in all groups during corresponding observation periods. There was however a definite change from early to late first stage of labour. The proportion of contractions of type II to contractions of type III was about 2:1 during early labour but changes to about 1:1 in the active phase. A change in this direction was observed in 26 of the 39 induction patients studied during both periods. Only 5 patients had a lower proportion of type III contractions during the active phase than during the latent phase.

Findings from pooled data seemed to indicate that atypical patterns were more common in prostaglandin induced or spontaneous labour than in labour induced by oxytocin. However Fig 3 shows that most patients in all three groups had a

very low incidence of atypical forms. A high proportion of atypical patterns was seen only in the recordings from a small number of patients in each group. There was a definite difference in this respect between primiparae and multiparae. Of 10 patients who had more than 5/20 (25%) atypical contractions during the active phase of labour 8 were primiparae. Three of the four PGF_{2α} induced patients in this category had atypical patterns during both the latent and active phases of labour. Conversely none of the oxytocin inductions showed this type of aberration during both phases. Examples of atypical labour patterns from one patient in each group are shown in Fig 4.

Relation of the intensity and the duration of the contraction to the shape of the pressure recording

The intensity and duration of contractions of type I, II and III were studied in 8 patients from Group O and 8 patients from Group P. It was found that the duration of the contractions irrespective of type was very constant in the same individuals in both groups of patients with coefficients of variation of 7–13%. This variation is of the same magnitude as the error of measurement. The mean duration of the contractions was 1.29 ± 0.15 minutes for the patients from the P group and for the patients in the O group 1.27 ± 0.14 minutes (means \pm S.D.). In all the 16 patients the intensity of the type III contractions was higher than for contractions of type II. The average ratio in intensity between contractions of type II and type III was 3:4 in both the O and P groups. Type I contractions had the lowest intensity but their number was small during the periods studied.

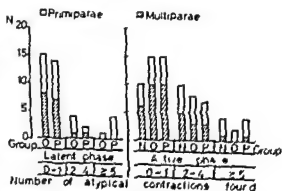


Fig 3 Number of parturients with different degrees of atypical uterine activity in the three groups of patients

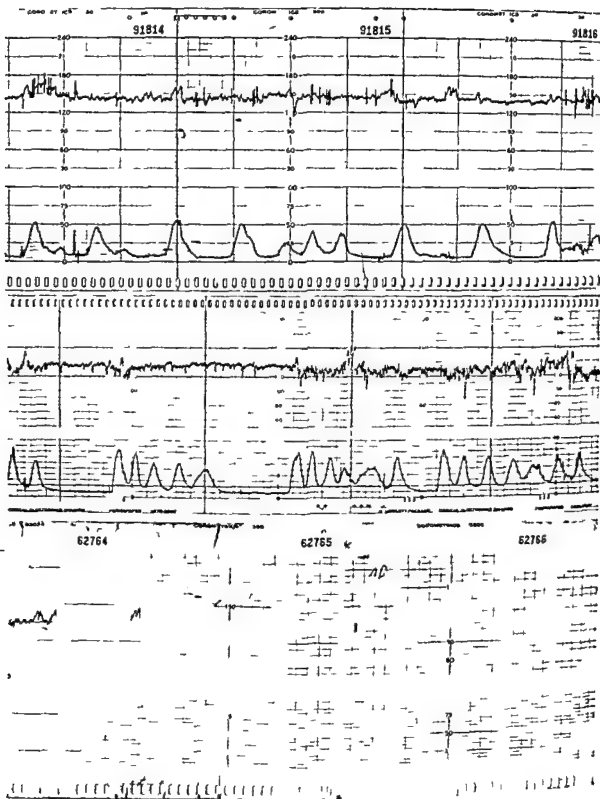


Fig 4 Examples of atypical uterine contraction patterns in 3 patients Top Spontaneous labour Middle Oxytocin induced labour Bottom PGF_α induced labour Upper recording Fetal heart rate beats per minute Lower recording Intra uterine pressure mmHg

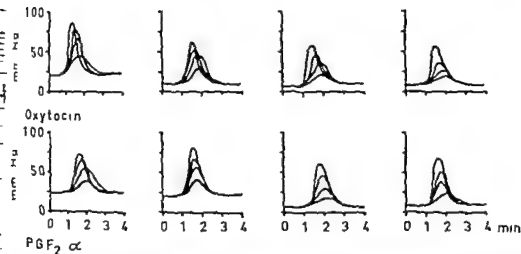


Fig 3 The shape of the uterine contraction at four different dose levels of the induction agent in 4 labours

induced with oxytocin (top) and $\text{PGF}_{2\alpha}$ (bottom) Oxytocin 2 4 8 12 mU/min $\text{PGF}_{2\alpha}$ 3 6 9 12 $\mu\text{g}/\text{min}$

The constant duration of the contractions and the change of the form of contraction during the course of labour with increasing intensity is also illustrated in Fig 5. Here the contraction patterns in the same patient at four dose levels of the infusion agent are illustrated. Details are given in respect of four patients from each of the induction groups. It is clearly seen that the upward slope of the intrauterine pressure wave became steeper as the amplitudes of the contraction increased with higher infusion rates of the oxytocic substance.

Effects of the shape of the contractions on the progress of labour

The maximum rate of cervical dilatation during the active stage of labour was calculated in all the 36 primiparae and related to the frequency of atypical contraction patterns found for each patient. It was found that the maximum rate was 7.3 ± 3.7 cm/hour for primiparae with 0–5% atypical contractions and 3.1 ± 1.9 cm/hour (means and S.D.) for those with 75% or more. The difference was statistically significant ($p < 0.01$). The total duration of the whole first stage in the induction cases was shorter in both primiparae ($0.10 > p > 0.05$) and multiparae ($0.05 > p > 0.01$) with 5% or less atypical contractions than in those with higher frequencies. There was no significant correlation between the proportion of type III contractions during the active phase and the speed of cervical dilatation.

Changes in fetal heart rate of the early deceleration type were observed in connection with more

than one or two contractions in 17 patients in the whole series. No significant differences in the occurrence of early decelerations were found between the groups. When early decelerations occurred they were often a rather constant finding in the same patient. They were clearly related to the intensity of the contraction. Approximately 75% of the early decelerations were found in association with contractions of 45 mmHg or more. The form of these contractions was almost exclusively type III. FHR-changes were only occasionally observed in connection with atypical contractions even when these occurred frequently (Fig. 4). Late or variable decelerations were seen as short episodes in 2 patients, probably caused by maternal hypotension due to compression of the vena cava in the supine position. Otherwise late decelerations or prolonged bradycardia (< 100 b.p.m.) were observed only in association with high frequency and intensity of contractions and raised basal tone in one patient in each of the O and P groups.

In a previous report on induction with $\text{PGF}_{2\alpha}$ (26) we found the fetal acidosis at birth to be more pronounced if labour had been incoordinated or included periods of hypertonus. Base deficit and pHi values in the umbilical artery from 16 cases with less than 5% atypical contractions were therefore compared to the values from 6 cases with 25% or more atypical patterns. BD_{AET} was 5.7 ± 2.8 mEq/l and 8.3 ± 2.5 mEq/l in the two groups and the corresponding pHi values 7.28 ± 0.06 and 7.22 ± 0.06 . The differences indicated a trend but did not

reach statistically significant levels when tested with Student's *t* test

The Apgar score in the 76 infants was 7-10 after 1 minute and 9-10 after 5 minutes

The O and P groups differed with respect to length of pregnancy and pelvic score at the start of the induction of labour. We could not find any association between a low pelvic score and the tendency to atypical uterine activity. Incoordination was not correlated to the dosage level of the oxytocic agent. In the patients with pronounced incoordination this tendency was mostly seen from the start of the infusion even at very low dosage levels

DISCUSSION

When Baumgarten (3) was working on this method of classifying the shapes of contractions he found that contractions with a steep upward slope of the pressure wave (type III) were seen with increasing frequency during the course of labour. In late first stage they constituted approximately two thirds of the contractions. This is in good agreement with our findings and with those of Tesch et al (36). Baumgarten suggested that this type of contraction is associated with good progress of labour and even that a low percentage might be an indication for stimulation with oxytocin. He claimed that oxytocin gives a higher proportion of these effective contractions. Our results indicate that the shape of the uterine contractions is related to the intensity

spective of the induction agent used. This agrees with the observation by Thorne et al (38) at the change of pressure with time (dPdT) is closely correlated to the amplitude of the contraction. It seems rather probable that a rapid rise in the intra uterine pressure would mean increased efficacy of the uterine activity. We could however not demonstrate any direct correlation between the proportion of type III contractions and the maximal speed of cervical dilatation during the active phase.

The tendency towards an incoordinated uterine activity during labour differs to a high degree between individuals. Quite clearly parity is an important factor. It is also true that primiparae are known to show dysfunctional labour more often than multiparae (6, 20). As the number of patients with a more pronounced incoordinated activity was rather small in our series it is not possible to form any definite conclusion about differences between

the two induction agents in this respect. Within the dose range used it can however not be concluded that prostaglandin administration conveys a greater risk of incoordinated labour. That the induction agent is not essential for the type of uterine activity found was also suggested by Tchilingirian (37) after trying both oxytocin and prostaglandins in the same individual.

It has been claimed by Jeffcoate (20, 39) that uterine incoordination might be caused by a high cervical resistance and this statement is supported by the fact that incoordination is found more often in primiparae. In consequence a more close association between incoordination and a short length of pregnancy and a low Bishop score would have been expected. This was not the case in this study and a possible explanation is that the series comprises only pregnancies around or at full term.

A high frequency of atypical contraction patterns is considered to result in slow progress of labour (1, 9, 23, 32). To a certain degree this was also true in our patients. It must be noted however that no patients with really prolonged labours or pronounced uterine inertia were included in the study. The progress of labour was in all cases within the normal range as defined by Friedman (15). Lindgren (23, 24) has proposed that the slow progress found in incoordinated labour might be mainly caused by the low intensity of many of the contractions in this type of labour. This was certainly true for some of our cases. It is obvious that a high cervical resistance and uterine incoordination often coexists. In those cases it is open to discussion which factor is then the main cause of a slow progress of labour.

As has been shown in several experimental studies both in animals and in humans (7, 27, 29) the uterine blood flow during labour is closely related to uterine activity. During the contraction the circulation through the placenta is reduced, probably mainly due to venous compression and stasis of blood in the intervillous spaces (2). Studies based on temperature changes show that the maximal decrease in the flow is seen during the downslope of the contraction (8) and that an adequate length of the relaxation period is necessary to reach basal flow again after the contraction (31). The normal monophasic contraction in our study had a fairly constant duration during labour which is in agreement with the results of Krapohl et al (21). The occurrence of prolonged atypical contractions as

defined previously will then result in a prolonged period of impaired circulation through the intervillos spaces. If a high frequency of contractions coincides with atypical labour patterns this danger will increase. In some cases however atypical complexes seem to be followed by a compensatory pause (Fig. 4) and this might give better chances for a return to a normal placental circulation.

When the fetal oxygenation is decreased below a critical level during labour the fetus will react with heart rate changes (4, 10). As discussed above the uterine contractions have a marked influence on the fetal oxygenation. Thus atypical contractions may be expected to provoke FHR changes. In fact it has been claimed that decelerations in the fetal heart rate occur with increasing frequency even in connection with a relatively prolonged downslope of the contraction as seen in the type III contraction (11). It has also been reported that with increasing intensity of the uterine activity early FHR decelerations are seen more frequently (32). This is also in good agreement with the findings in various experimental studies (27, 28). In our study early decelerations were seen mainly in connection with contractions of rather high intensity but no difference between patients with induced and spontaneous labour was found which is in agreement with several earlier reports (18, 40). As FHR decelerations were only occasionally seen together with the atypical contraction patterns in our study it could be concluded that these generally are not the cause of such increased strain to the fetus as might be expected. A tendency to a more marked acidosis was however found in the infants of mothers with atypical contractions.

The results of the present study do not support the view that oxytocin always gives rise to more regular contraction patterns in comparison to those found in spontaneous labour or following induction with PGF₂. Oxytocin has sometimes been recommended as a suitable therapeutic agent for the treatment of incoordinate labour (6, 23, 40). This type of treatment may possibly be successful in some cases but the infusion of an oxytocic agent in the presence of incoordinated labour implies a definite risk that the substance may cause an increase in the frequency of the contractions resulting in inadequate relaxation periods (35). A successive increase of the basal tone level could also give rise to a tetanic contraction with risk of fetal asphyxia.

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RADIOISOTOPE RADICAL OPERATION FOR CANCER OF THE CERVIX¹

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Abstract By performing the radioisotope radical operation (pre surgical labelling of the lymphatic tissue of the pelvis with colloidal ¹⁹⁹Au) with the aid of a gamma camera it was possible in 78 out of 93 cases to completely extirpate the regional lymphatic tissue of the pelvis. In eight out of 24 cases with positive findings on the lymph nodes we were also able to extirpate with this technique residues of carcinomatous lymphatic tissue during the course of a secondary isotope lymphadenectomy. This fact is likely to further improve the recovery rate—all the more so since even the scanner and eye probe technique already reduced the mortality rate due to recurrence by 16.4% after 5 years.

Ever since the introduction of mandatory lymphadenectomy in abdominal radical operations for cervical carcinoma it has been generally assumed that a complete extirpation of the regional lymphatic tissue is not possible. Efforts to label regional lymph nodes with lipiodol (1, 2, 3, 13, 14) and dyes (12, 15) were only of partial success. This method did not allow to detect and extirpate nodes which were concealed by blood vessels and ligaments. In order to tackle this drawback the radioisotope operation for cancer of the cervix uteri has been developed. The surgical procedure consists in pre operative radioactive colloidal gold labelling of the lymphatic tissue in the pelvic region.

MATERIALS AND METHODS

To this end 100 μ Ci colloidal ¹⁹⁹Au per extremity are administered subcutaneously between the first and second toes 4 hours before the operation. During surgery labelled lymphatic tissue may be scanned and extirpated, thus significantly enhancing the radical nature of the lymphadenectomy. In conventional surgery this lymphatic tissue due to lack of visibility cannot be removed.

The radioisotope radical operation has undergone three phases of development. In the first one a Magna Picker Scanner 3 was used in order to pinpoint radioactively labelled tissues not removed as yet. In phase 2 the technique was improved by introducing an eye probe (4, 5, 6, 7). Success was underlined by fact that in 4 out of 10 cases of carcinomatous lymph nodes extirpation of tumour tissue residues was possible in the course of a secondary specific intervention. At this particular site a recurrence might be avoided. The mortality rate due to recurrence was thus lowered to 16.4% five years postoperatively (Table I); this is an improvement compared to the results of the conventional Wertheim radical operation. Nevertheless the figures did not quite conform to our expectations since in only 57.6% of all cases operated during the years 1969/1970 and 1971 it was possible to extirpate completely the labelled lymphatic tissue.

For the above mentioned reason we decided to try and develop phase 3 of the radioisotope radical operation—introducing a surgical gamma camera that we had designed ourselves (Fig. 1). Since the beginning of 1972 this camera has been in use at our clinic (Fig. 2) (8, 9).

The fundamental difference between the scanner and eye probe methods on the one hand and the camera technique on the other hand may be described as follows: with the scanner and eye probe the radioactively labelled tissue

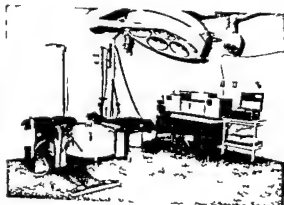


Fig. 1 The surgical gamma camera unit

¹Presented in part at the Thirteenth Congress of the Pan Pacific Surgical Association Honolulu Hawaii Feb 1975.

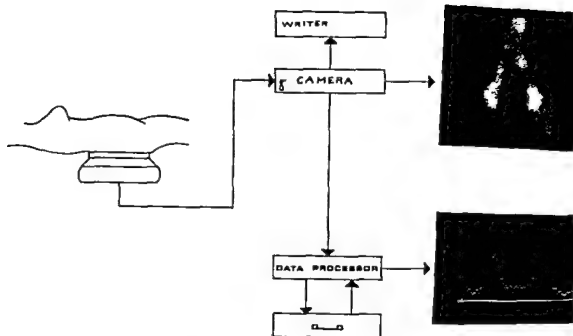


Fig 2 Scheme of the surgical gamma camera unit with oscilloscope and profile pictures

has to be scanned with a 3 in crystal across a large area whereas the use of the camera permits the entire field of surgery to be included in the crystal field of the detector head—thus making possible an overall image of the radioactively labelled tissue on the camera's oscilloscope screen. During this procedure the patient does not lie on a conventional operating table but on a specially adapted collimator surface of the gamma camera (Fig 2). The

entire pelvis is positioned on the crystal disc (30 cm in meter) of the camera's detector head. Since the top of the operating table is positioned on the left side of collimator disc (when viewed from the tripod) the th and arm supports are attached on the same side administering anesthesia. To the right of the tripod

Table I Recurrences in conventional and radio-isotope radical operations

Period of	No of cases	Death by recurrence	Mortality could be reduced by
Group I			
Wertheim surgery			
1965	61	9	
1966	44	5	
1967	49	9	
Total	154	23=14.93%	16.38% ~16.4%
Group II			
Radio isotope radical operation with colloidal ¹⁹⁹Au			
Nov 1969 until March 31 1971	40	5=12.5%	

In Group I 65.1% of cases were found at stage I
In Group II 67.5% of cases were found at stage I

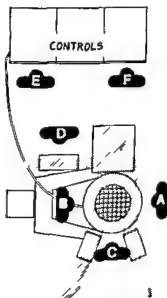


Fig 3 Scheme of the surgical gamma camera unit during the operation A=surgeon B=first assistant D=anesthetist E and F

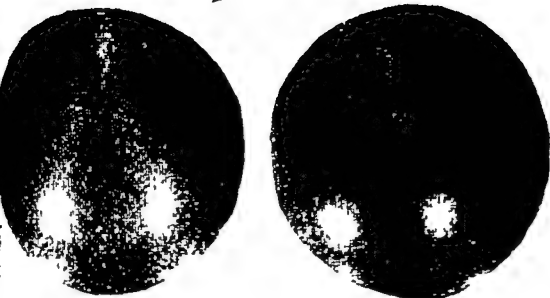


Fig 4 Oscilloscope picture before primary lymphadenectomy (left) and after surgery (right)

supports are attached in a way resembling a Wertheim operating table—with the second assistant standing between the legs of the patient. The surgeon works at the side of the detector head where there are not other attachments; the first assistant facing him, i.e. he stands between the U shaped struts of the support (Fig. 3).

24 hours after labelling with radioactive gold the radical operation according to Latzko-Wertheim-Meigs is performed: lymphadenectomy being carried out before removal of the surrounding tissue. Following the classical extirpation of the internal genitals including the surrounding paratissue and primary mandatory lymphadenectomy the residual lymphatic tissue is visualized on the oscilloscope (Fig. 4).

The secondary isotope lymphadenectomy is performed by a second and well rested surgical team, since such an operation requires utmost concentration. The preoperative lymphscan is made to coincide with a coordinate grid conforming to the oscilloscope screen of the camera. Certain squares on this screen are assigned to the lymphatic tissue areas of the scintigram. We call them 'areas of interest' with activities to be visualized as profiles; they may be counted with the camera's digital unit and recorded with a polaroid camera. There is three-dimensional recording—a top view on the oscilloscope screen, a profile view in the 3rd dimension and a depth view. If there is no chance to extirpate all affected lymphatic tissue with this grid technique, there is still the possibility of locating residual labelled tissue with a cobalt rod (^{60}Co) (Fig. 5). The luminous spot produced by this cobalt rod on the screen of the oscilloscope is sited to coincide with the residual lymphatic tissue; at this point the specific area has to be vertical to the cobalt rod in either a dorsal or a ventral direction. Thus we are always able to locate and extirpate

tissue even if it is concealed by major vessels or ligaments.

This surgical intervention is completed once the profile of activity recorded over the respective lymphatic tissue area does not exceed basic pulse values (Fig. 6). Subsequently quadruple abdominal drainage is performed, one redon drain being inserted on either side into pararectal fossa and the obturator fossa. Then peritoneum and abdominal wall are closed. Postoperative treatment is performed according to the Vienna method (10, 11).

RESULTS

Up to now 93 subjects have been operated on with the aid of the gamma camera with radioactive colloidal gold labelling prior to surgery. Complete ex-



Fig 5 Cobalt rod (^{60}Co) which is used for detection of radioactivity

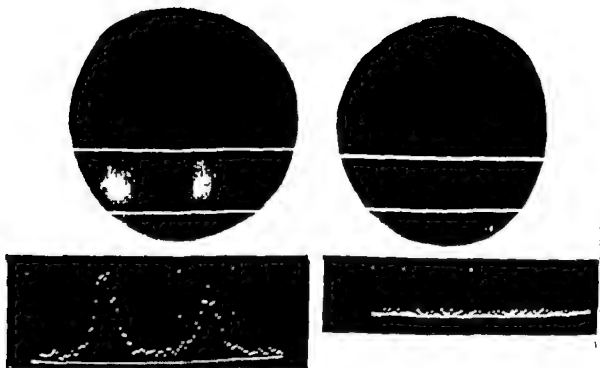


Fig 6 Oscilloscope and profile pictures before secondary lymphadenectomy showing residual lymphatic tissue (left) and after secondary isotope lymphadenectomy showing that the pelvis is cleaned up (right)

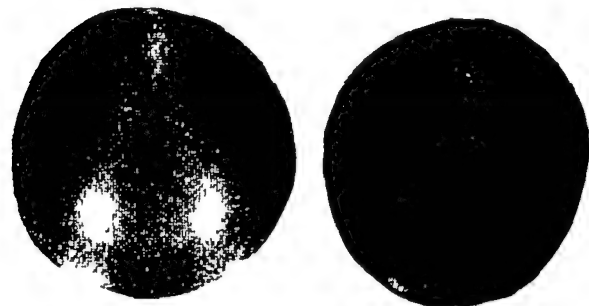


Fig 7 Oscilloscope picture before radioisotope radical operation showing ^{199}Au labelled lymphatic tissue (left) No labelled lymphatic tissue can be visualized after surgery except for the upper part of the inguinal lymph nodes (right)

Table II *Gammacamera method and capacity of lymphadenectomy*

No. of cases	Lymphadenectomy	
	Complete	Not complete
100	78=83.8%	15=16.2%

removal of the labelled tissue was possible in 78 patients (83.8%) (Fig. 7 and Table II). This improved technique of lymphadenectomy proved to be particularly valuable in cases with gland involvement. In eight out of 24 cases with histologically confirmed positive lymph nodes additionally affected could be extirpated within the scope of a lymph node specific lymphadenectomy. All groups of lymph nodes in all 93 patients and even those with carcinoma invasion could be detected. No radioactive gold is taken up by those lymph nodes which are completely invaded by the carcinoma. But in such cases the nodes are already enlarged and palpable and therefore often surgery is impossible in these subjects. There was no such a case among our patients. This fact is likely to result in further improvement of the statistics since—as mentioned above—a 16.4% reduction of the recurrence mortality rate had already been recorded for stages I and II over a five year period.

COMMENTS

Statistics demonstrating the absence of mortality and the unchanging morbidity level (ileus 0, mortality 0, thrombosis 0, peritonitis 0, ureter fistula frequency 1.07%, no other fistulae) encouraged us to report our technique in radioisotope radical operations.

Because of the expected improvement of the recovery rates subsequent to surgical treatment of cervical carcinoma we would like to submit this method for scrutiny.

In all cases mentioned above radioactively labelled tissue could be removed during the secondary radioisotope lymphadenectomy and in some 30% of lymph node positive cases additional cancerous lymph glands were found. This clearly shows that the usual lymphadenectomy which has been known for a long time to be not radical enough can be considerably improved by this method.

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SHORT COMMUNICATION

SERUM LEVELS OF THE PREGNANCY ZONE PROTEIN DURING THE NORMAL MENSTRUAL CYCLE

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The induction of the immunosuppressive serum protein PZ (pregnancy zone protein) caused by oestrogens is well known. Increased levels of PZ are found during pregnancy, during treatment with oral contraceptives and conjugated oestrogens and in males treated with oestrogens for prostatic cancer (1-4). The recent development of a sensitive radioimmunoassay for this protein has made it possible to measure the low basic levels and age-dependent increase in serum PZ concentration present in males and in non-pregnant healthy females (5, 6). Due to the oestrogen-dependent induction of PZ synthesis it could be expected that

the variation in endogenous oestrogen levels during the menstrual cycle would influence the PZ levels. The present communication describes simultaneous measurement of PZ, low polar oestrogens, progesterone, LH and FSH during normal menstrual cycles.

EXPERIMENTAL

Serum samples were obtained from 12 apparently healthy women of various ages during their menstrual cycle. Five of these women were followed with daily samples. The sera were stored at -20°C until analysis. Each sample was coded and tested blindly in duplicate. Serum levels

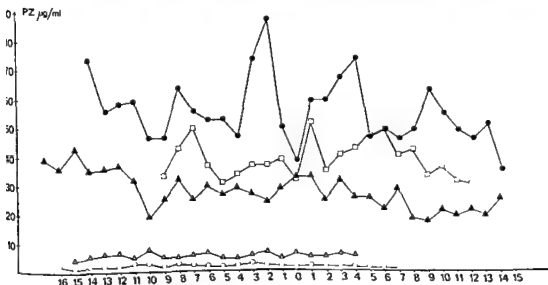


Fig 1 Serum levels of PZ in five complete menstrual cycles. Ovulation is indicated by day 0.

SHORT COMMUNICATION

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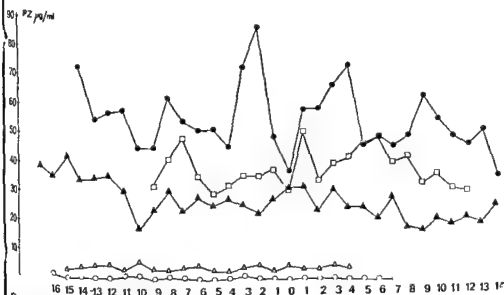


Fig 1. Serum levels of PZ in five complete menstrual cycles. Ovulation is indicated by day 0.

of PZ, FSH, LH, low polar oestrogens (LPE i.e. immunoreactive oestrogens) and progesterone were determined by radioimmunoassay techniques as previously described (5-11).

RESULTS AND COMMENTS

The levels of PZ during five complete individual cycles are shown in Fig. 1. No significant pattern in the PZ levels could be found and there was no statistically significant correlation between the levels of PZ and of any of the hormones. The PZ values ranged between 2-80 µg/ml and as has been shown previously the higher values were present in the older women (6). There was a pronounced intra-individual variation in the PZ levels. The results indicate that the peak values of oestrogens during the normal menstrual cycle are not of sufficient magnitude or duration to induce increased serum levels of PZ. There are still no basic data available concerning lag time and threshold levels for the oestrogen induced synthesis of PZ.

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- Antibody against progesterone 11 α -hemisuccinate BSA, raised in sheep (Royal Veterinary College, Stockholm, Sweden). Dextran coated charcoal separation.

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Letter to the Editor

Dear Sir!

We have studied with great interest a recent article published in *Acta Obstet Gynecol Scand* by Drs Maltau and Andersen. Continuous epidural anesthesia with a low frequency of instrumental deliveries (*Acta Obstet Gynecol Scand* 54 401 1975). As their results and conclusions are somewhat different from ours (1) we would like to make the following comments.

The authors main point is that epidural analgesia in labour will not increase the frequency of instrumental deliveries. In the introduction they refer to two studies (3, 6) in which the same conclusions were made. It should therefore be noted that the frequency of instrumental deliveries in the actual studies were Doughty 42% for primipara and 11% for multipara while Potter reported 25-30% in instrumental deliveries in primiparae. In comparison with Maltau & Andersen's as well as with our own (1) control group without epidural block these figures are still relatively high.

We do anyhow also think that the frequency of instrumental deliveries after epidural block can be kept at an almost normal figure. This will however lead to an increased duration of the second stage of labour. Crawford, a leading authority with in this field as well as Potter, which were referred to in Maltau & Andersen's study have noted this increased duration of the second stage of labour (2, 6). Crawford also does advocate an increased frequency of instrumental deliveries to counteract this phenomenon.

The second stage of labour imposes an increased stress on the fetus and should not be delayed. Acid-base studies (4, 8) have clearly demonstrated this.

We think that the neonate is better off after an instrumental delivery and a normal second stage than after a prolonged second stage and a spontaneous delivery. When using epidural block in labour a normal frequency of instrumental deliveries is not in the best interest of the newborn.

Maltau & Andersen state in their discussion that the second stage never exceeded 1 hour. This figure seems questionable when for example Crawford's figures regarding the duration of the second stage after epidural block in about 1000 patients are studied (2). In our own experience a frequency of instrumental deliveries of at least 40% in primiparae is needed to keep the second stage to approximately 1 hour. Did Maltau & Andersen use fundal pressure and episiotomy in all patients?

The figures reported by Maltau & Andersen regarding the doses per hour of Bupivacaine are rather unusual. Bupivacaine was injected approximately every hour (every 45 min in multiparae) which is probably the shortest dose interval published for 0.25% Bupivacaine (with or without adrenaline). Previous studies (1, 2, 7) have demonstrated a dose interval of approximately 2 hours which is one of the main advantages of Bupivacaine while dose intervals of approximately 1 hour are mainly seen with shorter acting agents like Lidocaine (8).

Finally it was noted that in 19 cases the indication to epidural block was intrauterine distress. This is a rather uncommon indication and it would be interesting to know how the diagnoses were made (continuous fetal heart rate monitoring or fetal scalp blood sampling) and in which way these parameters changed after the initiation of an epidural block.

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of PZ, FSH, LH, low polar oestrogens (LPE, i.e. immunoreactive oestrogens) and progesterone were determined by radioimmunoassay techniques as previously described (5-11).

RESULTS AND COMMENTS

The levels of PZ during five complete individual cycles are shown in Fig. 1. No significant pattern in the PZ levels could be found and there was no statistically significant correlation between the levels of PZ and of any of the hormones. The PZ values ranged between 2-80 $\mu\text{g/ml}$ and as has been shown previously the higher values were present in the older women (6). There was a pronounced intra-individual variation in the PZ levels. The results indicate that the peak values of oestrogens during the normal menstrual cycle are not of sufficient magnitude or duration to induce increased serum levels of PZ. There are still no basic data available concerning lag time and threshold levels for the oestrogen induced synthesis of PZ.

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